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An Assay to Quantify Methylphenidate and Atomoxetine in Pharmaceutical Preparations by Micellar Liquid Chromatography

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ABSTRACT

We developed a practical and economic method based on micellar liquid chromatography for the quantification of atomoxetine and methylphenidate, two central nervous system stimulants used to treat attention deficit hyperactivity disorder, in solid dosage forms. Samples were solved in mobile phase and directly injected, after filtration. Both drugs were resolved from matrix compounds using a carbon-18 column and a mobile phase consisting in a solution of 0.10 mol/L sodium dodecyl sulfate—6% 1-pentanol, phosphate-buffered to pH 7, running at 1 mL/min under isocratic mode. Detection was by absorbance at 210 nm. The effect of the surfactant and the organic modifier on the elution strength of the mobile phase was established for theoretical purposes and to expedite the optimization step, using a chemometric approach. The procedure was successfully validated by the guidelines of the International Council of Harmonization in terms of selectivity, linearity (determination coefficient > 0.995), calibration range (1–10 mg/L), trueness (99.6%–102.8%), precision (< 2.8%), and robustness, and it was applied to measure the amount of drugs in commercial presentations. The method exhibited adequate analytical performances, used a low amount of harmful and pollutant reagents, required available laboratory material, and displayed an adequate sample throughput; thus, it was useful for routine analysis.

1 | Introduction

Atomoxetine (ATX) and methylphenidate (MPH) are central nervous system (CNS) stimulants indicated and Food and Drug Administration (FDA)-approved for the treatment of attention deficit hyperactivity disorder (ADHD) in adults and children over six years [1–3]. ADHD is a psychiatric dysfunction that causes inappropriate levels of inattention, hyperactivity, and impulsive behavior in patients. The symptoms begin at a young age but may persist in adulthood. Patients suffering from ADHD can have difficulty with social interactions and completing tasks, which impair their academic, social, and job functioning [4–6]. It has

Abbreviations: ADHD, attention deficit hyperactivity disorder; ATX, atomoxetine; CNS, central nervous system; FDA, Food and Drug Administration; HMLC, hybrid micellar liquid chromatography; ICH, International Conference of Harmonization; *k*, retention factor; LOD, limit of detection; LOQ, limit of quantification; MPH, methylphenidate; *N*, number of theoretical plates; *p*, *p* value; *Po/w*, partition coefficient between octanol and water; QC, quality control; R^{2}, adjusted multiple determination coefficient; r^2, determination coefficient; R^2, multiple determination coefficient; RP, reverse phase; RSD, relative standard deviation; SD, standard deviation; SDS, sodium dodecyl sulfate; *T*, tailing factor; t_R, retention time; UV, ultraviolet.

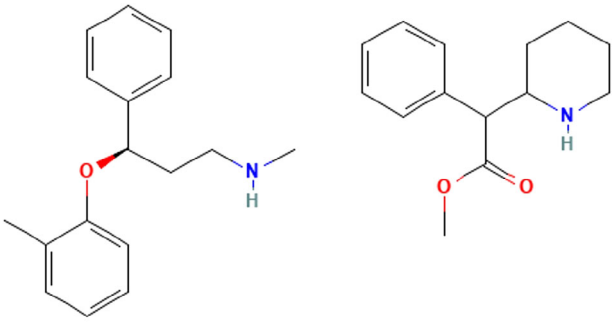

FIGURE 1 | Structure of both analytes.

an estimated worldwide prevalence of nearly 10% in school-age children [5].

ATX ((3R)-N-methyl-3-(2-methylphenoxy)-3-phenylpropan-1-amine) is a nonstimulant selective, presynaptic, norepinephrine reuptake inhibitor antidepressant. It inhibits the presynaptic norepinephrine transporter, preventing the reuptake of norepinephrine throughout the brain and inhibiting dopamine reuptake in specific brain regions. ATX is well-absorbed after oral administration (bioavailability 63%–94%) and is minimally affected by food. It is administered as its HCl salt in capsules ranging from 10 to 100 mg and taken, with or without food, as a once-daily or two-evenly divided dose. It is also used against adult patients with treatment-resistant depression [1, 3, 5]. Chemically, it is a substituted aromatic ether, a secondary amino compound and a toluene with a molecular mass of 255.4 g/mol (small molecule). It is moderately hydrophobic (log $Po/w = 3.9$) and alkaline with a pKa of 10.1, corresponding to the protonation of the amino group. Its formal charge is +1 in pH values between 0 and 7.5 [3, 7]. Its structure can be seen in Figure 1 [7].

MPH (methyl 2-phenyl-2-piperidin-2-ylacetate) is a CNS stimulant. It inhibits the reuptake of two neurotransmitters, norepinephrine and dopamine, in presynaptic neurons, thus, extending its activity in the synaptic cleft. It is administered as its hydrochloride salt mainly by the oral route (bioavailability of nearly 11%–52%), as chewable and immediate and extended released tablets containing doses from 2.5 to 85 mg, and less commonly, as a transdermal patch. It is also used to treat narcolepsy, fatigue in patients with cancer, refractory depression in the geriatric population, and apathy in Alzheimer's disease and to improve cognitive performance [2, 3, 6]. Chemically, it is a small molecule (233.3 g/mol) and belongs to the categories of piperidines, methyl esters, and beta-amino acid esters. It is moderately hydrophobic (log $p = 2.3$) and basic, with a pKa of 9.0 (ionization of the piperidine group) and its formal charge is +1 between 0 and 7.5 [3, 7]. Its structure can be seen in Figure 1 [7].

Clinical effects of both drugs depend on the physiology of the patient and require dose individualization [5, 6]. Typical daily doses range from 40 to 100 mg for ATX [1] and from 5 to 60 mg for MPH [6]. In addition, patients require a careful follow-up to evaluate treatment efficacy, tolerability, and correct adherence. Thus, all patients should be monitored for their vital signs and for emergence of other psychiatric disorders. Special attention should be paid to the cardiovascular system, especially in patients with previous blood-related disorders. In pediatric patients, it is also essential to evaluate their growth. Dose adjustment or discontinuations may be considered as a result of these regular visits [1, 2]. ATX is less effective and may take several weeks for full noticeable therapeutic action than MPH but has less risk of addiction and adverse effects. These two drugs are not coadministered [1, 3]. However, their simultaneous determination can be useful for pharmaceutical laboratories controlling oral dosage forms containing either ATX or MPH, so that they can indistinctly analyze them without a need of changing the instrumental conditions, which favors and simplifies the laboratory work and then increases sample throughput.

Reverse phase liquid chromatography is the most employed tool for drug determination for pharmaceutical purposes because of its versatility, analytical performances, and easy automation [8]. Hydro-organic reverse phase (RP)-HPLC with ultraviolet (UV) absorbance detection has been used to determine ATX in dosage forms, alone [9–15], with its impurities [16], and with other drugs [17], as well as MPH and its impurities in bulk [18] and pharmaceuticals [19, 20]. Columns used were carbon-8 [10, 12, 14] and carbon-18 [9, 11, 15–20], and mobile phases were mixtures of an aqueous buffer (pH 3.0–6.8) and variable proportion of volatile and toxic organic solvents (5%–82%), such as acetonitrile [9, 11–20] and methanol [10, 16, 18, 20], running under isocratic mode. Strategies applied to reduce the interaction of the cationic analytes with the free silanols were the adjustment of pH to 3 [12, 14, 15, 17–19], the addition of triethylamine as a sacrificial base [11, 14, 19], and by ion-pairing [19]. Dosage forms were mainly solved in mixtures of methanol/water [9, 10, 12], acetonitrile/water [11, 19], and methanol/acetonitrile/water [20] with proportions of organic solvent ranging from 50% to 100%, to achieve the solubilization of the active ingredient. As far as we know, ATX and MPH have not been previously determined together by HPLC. Although these two drugs cannot be found in the same dosage form [3], a method to their simultaneous determination may be useful in quality control laboratories receiving products containing either one or the other, to avoid cross-contamination. Moreover, the development of procedures minimizing the use of hazardous and pollutant chemicals is also a requirement for modern laboratories [21].

Hybrid micellar liquid chromatography (HMLC) is a branch of RP-HPLC that uses a C-18 column and solutions of sodium dodecyl sulfate (SDS) over the critical micellar concentration and a low proportion (< 15% v/v) of an organic solvent as mobile phases and as solubilizing solutions of the sample. This has been proven as a green alternative to determine drugs in pharmaceutical formulations [22–24], including antidepressants [25]. In general, the micellar environment extends the solubilization power of the solution to moderately hydrophobic compounds, which allow to reduce the proportion of organic solvent [26]. Besides, the adsorption of the monomer on the stationary phase, with the sulfate group oriented to the mobile phase, expedites the elution of cationic compounds by increasing their retention and impairing their interaction with the free silanols [27]. Otherwise, the retention mechanism is quite reproducible and can be modeled by well-known equations, which favor the optimization [28].

The aim of the research was the development of a safe, green, easy-to-conduct, and practical method to determine ATX and MPH, prescribed against ADHD in oral dosage forms by micellar liquid chromatography. In addition, the effect of mobile phase composition on retention will be studied as part of the optimization and for theoretical purposes. The evaluation of the analytical performances of the procedure will be carried out following the guideline Validation of Analytical Procedures: Text and Methodology Q2(R1) from the International Conference of Harmonization (ICH) [29], and finally, it will be applied to commercial formulations.

2 | Experimental

2.1 | Standard and Reagents

Powdered solid standards of ATX hydrochloride (pharmaceutical secondary standard) and MPH hydrochloride (certified reference material) were from Sigma Aldrich (Saint Louis, MO, USA). SDS (purity > 99%) was bought from Scharlab (Barcelona, Spain). Sodium dihydrogen phosphate monohydrate (> 99%), hydrochloric acid (37%), sodium hydroxide (99%), and the solvents (HPLC grade) methanol and 1-pentanol came from Panreac (Barcelona, Spain). Ultrapure water was produced in lab from deionized water (supplied by the university as tap water) using a water purification system Adrona HPLC Connect (Adrona, Riga, Latvia). All aqueous solutions were prepared using this water.

An analytical scale SAR CP224S Sartorius AG (Sartorius AG, Göttingen, Germany) was used to weigh reagents, standards, and samples. Ultrasonic bath was Elmasonic S 15 H (Elma, Frechen, Germany). A potentiometer GLP 22 Crison (Barcelona, España) equipped with a glass electrode and a Ag/AgCl reference electrode was used for pH measurements.

Micellar solutions were prepared as indicated in [30]. Stock solutions of ATX and MPH (100 mg/L) were prepared by weighing the proper mass and solving it in methanol. Working solutions were prepared by successive dilution of the stock solutions in mobile phase. These solutions were stored in amber vials in a fridge at +4°C.

2.2 | Chromatographic Equipment and Conditions

A HP100 system (Agilent Technologies, Palo Alto, CA, USA) equipped with a degasser, a quaternary pump, an autosampler, a column compartment, and a UV-absorbance diode array detector (DAD), connected to a PC, was used for the chromatographic analyses. The software Agilent ChemStation (Rev. B.04.03) was employed to control the instrumentation, as well as to register and visualize the signals. The working and cleaning specifications for chromatographic instrumentation when dealing with micellar mobile phases can be seen in [31].

The column was a Kromasil C-18 (150 × 4.6 mm; particle size, 5 µm; pore size, 10 nm) from AkzoNobel (Amsterdam, the Netherlands). The mobile phase was an aqueous solution of 0.10 mol/L SDS—6% 1-pentanol, buffered at pH 7 with 0.01 mol/L sodium dihydrogenphosphate. It runs under isocratic mode at 1 mL/min, without controlling the temperature. Injection volume and absorbance detection wavelength were 20 µL and 210 nm, respectively. The experimental dead time was 0.8 min. Retention factor (k), efficiency (number of theoretical plates, N), and asymmetry (tailing factor, T) were calculated as indicated in [32].

The solutions to be injected were filtered through a 0.45-µm nylon membrane filter (Micron Separations, Westborough, MA, USA) by hand pushing using a 3-mL syringe, then being introduced in respective chromatographic vials. Injected solutions were discarded.

2.3 | Sample Preparation

Some oral dosage forms containing the studied drugs as unique active component were randomly purchased from local chemists.

One solid form was grinded into fine powder using an Agate mortar and homogenized. An aliquot (depending on the expected quantity of active form) was introduced in a small beaker, and 10 mL of methanol and 30 mL of mobile phase were added. The mixture was shaken by magnetic stirring and ultrasonication to achieve drug solubilization (15 min each) and transferred to a 50-volumetric flask, which was filled up with mobile phase. These solutions were finally stored in 50-mL amber vials. Afterward, the appropriate volume was diluted in mobile phase to obtain a solution with a concentration of the active component close to the target concentration.

Samples and concentrated sample solutions were kept at +4°C in the fridge, while diluted sample solutions were discarded after analysis.

3 | Results and Discussion

3.1 | Optimization of the Chromatographic Conditions

A C-18 silica column, SDS as surfactant, isocratic mode, and a low rate of 1 mL/min were selected for the chromatographic elution, as these are the most common in HMLC for the analysis of drugs [33].

ATX and MPH display a formal charge of +1 in the entire working pH range of the column (2.5–7.5). Therefore, no significant influence of pH on retention is expected, and then it was adjusted to 7 to avoid acidic conditions, which may cause long-term column degradation. An organic solvent is usually added to the mobile phase to reduce its polarity and viscosity and then increase efficiency and elution power. Considering the high hydrophobicity of ATX and its positive charge, a high retention is expected, and then 1-pentanol was chosen as 1-propanol and 1-butanol would provide excessively long retention times (t_R). This would also be useful to elute MPH, with a moderate hydrophobicity [34]. Therefore, experimental assays were carried out to optimize the concentration of SDS and 1-pentanol and the detection conditions.

TABLE 1 | Retention time of the studied drug at the assayed mobile phases.

No.	[SDS] (M)	[1-pentanol] (%, v,v)	t_R methylphenidate (min)	t_R atomoxetine (min)
1	0.05	2	23.97	65.50
2	0.05	4	13.05	28.97
3	0.05	6	8.59	16.82
4	0.10	4	9.50	23.34
5	0.10	6	6.02	11.09
6	0.15	2	13.20	33.62
7	0.15	6	5.41	10.67

3.1.1 | Influence of the Mobile Phase Composition on Retention

Standard solutions of MPH and ATX 5 mg/L were analyzed by seven mobile phases selected by a full factorial design plus three additional points. The extreme values were the minimal and maximal concentration of SDS (0.05/0.15 M) and 1-pentanol (2%–6%, v/v) recommended for HMLC [35]. Results can be seen in Table 1.

We notice ATX is far more retained than MPH using any mobile phase, as expected by its higher hydrophobicity. Therefore, any issue regarding the resolution of both solutes is expected. Otherwise, the retention factor of both compounds decreases at higher concentration of SDS, and then number of micelles, pointing to a binding behavior between the two drugs and the micelles, probably mainly by electrostatics interaction. In addition, the elution strength of the mobile phase increases by augmenting the proportion of alcohol, as usually occurs in HMLC.

3.1.2 | Modeling of Retention Factor

Retention factor (as predicted variable) was modeled on SDS and 1-pentanol concentration (as predictor variables). Equation (1) has been demonstrated to be enough accurate in HMLC when employing a C-18 column saturated with SDS and a mobile phase running under isocratic mode for moderately hydrophobic drugs [33, 34]:

$$1/k = c_0 + c_1\,[\mathrm{SDS}] + c_2\,[1\text{−pentanol}] + c_{12}\,[\mathrm{SDS}]\,[1\text{−pentanol}] \quad (1)$$

where c_0 stands for the elution strength of the mobile phase at intermediate conditions, while c_1, c_2, and c_{12} stand for the influence of SDS, 1-pentanol, and the interaction, respectively. The relative influence of each factor regarding the elution strength at central conditions (c_x/c_0) can be used to compare between different drugs. The model can be constructed by performing a few experimental assays and can be used to estimate the retention factor of a drug at intermediate concentrations of SDS and 1-pentanol, without need of performing the chromatographic run. Besides, it permits the quantitative appraisal of the influence of SDS and 1-pentanol on retention. However, for highly hydrophobic compounds, such as ATX, an additional term describing the quadratic effect of the alcohol proportion is often required [33, 34]:

$$1/k = c_0 + c_1\,[\mathrm{SDS}] + c_2\,[1\text{−pentanol}] + c_{12}\,[\mathrm{SDS}]\,[1\text{−pentanol}] + c_{22}[1\text{−pentanol}]^2 \quad (2)$$

In order to find which model applies for each compound, the equations were adjusted using the experimental data in Table 1 with concentrations normalized from −1 to +1 by curve-fitting least-square regression. For MPH, the adjusted multiple determination coefficients (R'^2) were 0.9753 and 0.9697 for Equations (1) and (2), respectively, while 0.9212 and 0.9251, respectively, for ATX. Therefore, the inclusion of the quadratic term significantly improves the model for ATX but not for MPH [36], as expected by their respective hydrophobicity. Therefore, Equation (1) was used to model MPH retention behavior and Equation (2) for ATX. The results are in Table 2. The constructed models were judged enough accurate, as the multiple determination coefficients (R^2) were >0.97 and the regression residual <15%.

For both drugs, the higher constant was c_0, pointing to the variation of SDS/1-pentanol concentration from the central values inside the studied interval, which do not excessively affect elution strength. This value was 2.6 times higher for MPH. The relative influence of each factor was alcohol > SDS > interaction for both solutes. The effect of 1-pentanol was higher for ATX than for MPH. Indeed, as it is more hydrophobic, its solubility in the mobile phase benefits more from the reduction of the polarity of the mobile phase. The relative influence of SDS is the same, for it is essentially by electrostatics and both compounds display the same charge. Finally, we noticed a small but positive interaction, which means the elution strength slightly augments when both SDS and 1-pentanol concentrations increase or decrease together from the intermediate values.

3.1.3 | Optimization of the Composition of the Mobile Phase

The mobile phase was selected by evaluation of the results obtained in the assay described in Section 3.1.1 (Table 1). Mobile phases 1, 2, 3, 4, and 6 were discarded, as the global elution time would be >15 min. Finally, 5 was preferred over 7, for the difference in retention time is minimal and to reduce the

TABLE 2 | Equation adjustment to model the retention factor for the two analytes.

Parameter	Methylphenidate	Atomoxetine
$c_0 \pm$ SD (p)	0.095 ± 0.003 (<0.01)	0.037 ± 0.005 (0.02)
$c_1 \pm$ SD (p)	0.026 ± 0.004 (<0.01)	0.010 ± 0.003 (0.09)
$c_2 \pm$ SD (p)	0.047 ± 0.004 (<0.01)	0.026 ± 0.003 (0.02)
$c_{12} \pm$ SD (p)	0.010 ± 0.004 (0.08)	0.005 ± 0.004 (0.32)
$c_{22} \pm$ SD (p)	—	0.007 ± 0.006 (0.39)
Residual/model freedom degrees	3/3	2/4
R^2	0.988	0.975
|error|(%)	<8.0	<12.0
c_1/c_0	0.28	0.28
c_2/c_0	0.50	0.69
c_{12}/c_0	0.11	0.13
c_{22}/c_0	—	0.18

Note: error = $(k_{predicted} - k_{experimental})/k_{experimental} \times 100$
Abbreviations: p, p value; R^2, multiple determination coefficient; SD, standard deviation.

concentration of salt in the mobile phase and the column pressure.

3.1.4 | Optimization of the Detection Conditions

The absorption spectra of both compounds were registered in time during the chromatographic run using the selected mobile phase, at the retention time, as well as at 50% and 5%, both fronting and tailing edge for peak purity studies. Both spectra exhibited a high band at 210 nm and a minor band between 250 and 260 nm. Therefore, the detection wavelength was set to 210 nm.

3.1.5 | System Suitability Testing

In order to appraise the appropriateness of the chromatographic instrumentation and the operational conditions to provide consistent response, a solution containing 5 mg/L of MPH and ATX was analyzed by six successive replicate injections under the selected conditions (0.10 mol/L SDS—6 % 1-pentanol—pH 7 and detected at 210 nm). The obtained chromatogram is shown in Figure 2. The following chromatographic parameters were calculated (MPH, ATX, and acceptance criteria [32]): retention time (6.02 ± 0.02, 11.09 ± 0.03, —), relative standard deviation (RSD%) of retention time (0.4, 0.3, <1), RSD% of peak area (0.6, 0.7, <1), capacity factor (6.53, 12.86, >2.0), and efficiency (2356, 2029, >2000). A good separation between both peaks was achieved. Therefore, the results of the system suitability testing were considered satisfactory.

3.2 | Method Validation

The procedure was validated by the guidelines of the ICH Validation of Analytical Procedures: Text and Methodology Q2(R1) [37] (especially developed to determine active components in pharmaceutical formulations) and Methods, Method Verification and Validation, FDA-ORA Laboratory Manual Volume II [38], in order to appraise the quality of the qualitative and quantitative results. Unless specified, the experiments were performed using standard solutions.

3.2.1 | Calibration Range and Linearity

Standard solutions of both drugs up to 10 mg/L were analyzed six times. An F test was performed to compare the smaller and higher variance obtained at each level, and the model was found homoscedastic. A first-grade equation was constructed to relate the average peak area (response variable) and the corresponding concentration (predictor variable) by the least-square regression method. The slope, y-intercept, determination coefficient, limit of detection (LOD) and limit of quantification (LOQ) (taking the standard deviation of the y-intercept as the standard deviation of the blank) are shown in Table 3.

An adequate linearity was found, as both determination coefficients (r^2) were >0.995, the relative residual standard deviations were <3.0%, and the residual plot displays a random normal distribution. In both cases, the y-intercept displays a low influence in the calculation of the concentration, as it accounts for less than 3.0% of the target concentration and its interval confidence includes 0. The linear interval was set to 1.5–10 mg/L for both compounds, thus, covering 80–120 of the target concentration. Besides, this value is largely over the LOQ.

3.2.2 | Trueness and Precision

These parameters were determined at 4.0, 5.0, and 6.0 mg/L (0.8×, 1.0×, and 1.2× of the target concentration, respectively), separately for each drug.

Six standard solutions, which contained the studied concentration, different from those used in the calibration experiment, were

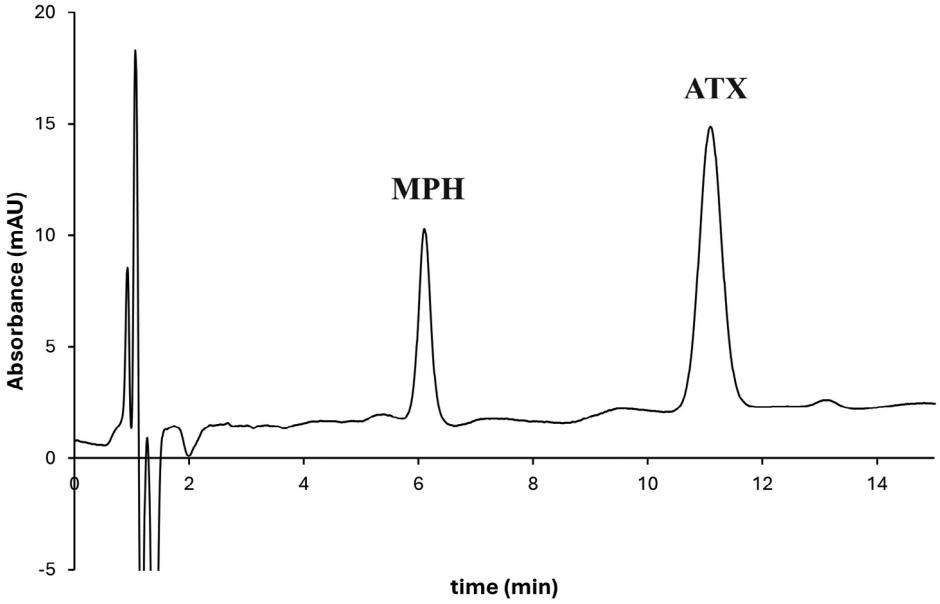

FIGURE 2 | Chromatogram obtained by the analysis of a mixture of methylphenidate and atomoxetine 5 mg/L under the optimal conditions.

TABLE 3 | Statistical parameters of linearity.

Parameter	Methylphenidate	Atomoxetine
Slope	25.4 ± 0.9	71.0 ± 0.9
y-intercept	4 ± 4	8 ± 5
r^2	0.9953	0.9993
LOD (mg/L)	0.5	0.2
LOQ (mg/L)	1.6	0.7
Relative residual standard deviation %	3.0	1.9
Weight of *y*-intercept on target concentration	3.0%	2.2%

prepared and analyzed consecutively. Trueness was expressed as the relative recovery, the quotient between the average found concentration and the nominal value, while the precision (repeatability) was the relative standard deviation of the results. The same experimental approach was performed five times over a 3-month period, using a renewed set of solutions each time, and the intermediate precision was calculated as the RSD% of all the calculated found concentrations. Results are shown in Table 4.

The values obtained were inside the acceptance criteria, thus, proving the reliability of the quantitative results. Besides, no replicate injections are required, which simplifies and cheapens the daily practice. These performances were attained because of the solubilization power of micellar solutions and the absence of a purification step.

3.2.3 | Carryover Effect

The absence of cross-contamination between samples and with standards is a mandatory requirement to achieve a reasonable accuracy for the quantitative results. A standard solution containing 15 mg/L of MPH and ATX and a blank standard solution were successively injected. No peak was noticed at or close to the window time of any of the studied drugs, thus, pointing out there is no noticeable carryover effect.

3.2.4 | Robustness

The robustness of the procedure was evaluated by the steadiness of retention time and peak upon slight variations of experimental parameters like SDS concentration (0.095–0.105 mol/L), 1-pentanol proportion (5.8%–6.2 %), pH (6.8–7.2), flow rate (0.95–1.05 mL/min), injection volume (19–21 µL), and registered absorption wavelength (205–215 nm) by a one-factor-at-a-time approach. Both chromatographic responses were measured (by the analysis of a standard solution of MPH and ATX 5 mg/L) at the optimal, maximum, and minimum values of each studied factor.

No significant alteration of retention factor or peak area was noticed for all the considered experimental parameters (RSD% of the three values < 5), and then the procedure provides consistent responses to minor fluctuations that can occur in the usual laboratory practice.

3.2.5 | Specificity

The capacity of the method to discriminate the signal of the drugs from excipients or impurities from the pharmaceutical preparation was evaluated by the analysis of an oral solid dosage form containing MPH and ATX, respectively, as unique active component, as no excipients were available. In the obtained chromatograms, no peaks were close to or were overlapping with those of the studied drugs. The chromatograms from the samples were compared by overlaying with those obtained from standard solutions (see Section 3.1.4), and the peaks of the drugs almost matched. Finally, a peak purity study was carried out by taking the absorption spectra at the same points as indicated in

TABLE 4 | Trueness and precision of the method (acceptance criteria: trueness, 98.7%–103.0%; repeatability, < 2.0%; intermediate precision, < 3.0%).

Drug	Concentration (mg/L)	Trueness (relative recovery, %)[a]	Repeatability (RSD%)[a]	Intermediate precision (RSD%)[b]
Methylphenidate	4.0	102.8	2.0	2.8
	5.0	101.4	1.6	2.1
	6.0	99.7	1.3	1.8
Atomoxetine	4.0	102.6	2.1	2.7
	5.0	101.4	1.9	2.3
	6.0	99.6	0.8	1.1

[a] $n = 6$.
[b] $n = 5$.

TABLE 5 | Comparison of this method to previously published ones.

	Atomoxetine		Methylphenidate	
	This work	[11]	This work	[18]
Organic modifier in mobile phase and proportion	6% 1-pentanol	Acetonitrile 58% + Triethylamine 0.03 mol/L	6% 1-pentanol	Acetonitrile 45% + Methanol 45%
Organic as sample solvent in mobile phase and proportion	6% 1-pentanol	Acetonitrile 58% + Triethylamine 0.03 mol/L	6% 1-pentanol	Methanol 100%
LOD	0.2	0.4 mg/L	0.5	1.5
LOQ	0.7	1.3 mg/L	1.6	4.5
Linear interval	1.5–10	0.4–40 mg/L	1.5–10	20–45 mg/L
Linearity (r^2)	0.9993	0.9994	0.9953	0.9981
Recovery	99.6%–102.6%	97.0%–104.3%	99.7%–102.8%	99.1%–99.8%
Precision	<2.8%	<1.1%	<2.7%	<0.9%
Retention factor	12.9	0.27	6.5	4.7
Duration of the run	15 min	3 min	15 min	8 min

Section 3.1.4 and visually comparing by overlaying with those registered from the analysis and were found rather similar.

We deduced that there are not any interfering compounds, and that the entire peak area can be assigned to the corresponding drug.

3.2.6 | Comparison With Previously Published Methods

The current method was compared to that in [11] and [18] to determine ATX and MPH, respectively, in oral dosage forms (Table 5). The proposed method here uses a less toxic and volatile organic solvent and at far less proportion for both mobile phase and sample solvent; thus, it is safer and more eco-friendly. The present method reaches lower values for LOD, LOQ, and linear interval. Moreover, the calibration range covers an order of magnitude slightly shorter than [11] and larger than [18], while linearity was alike. No significant difference was found for recovery and precision; hence, the quality of the quantitative results was similar. Retention factor for ATX was too low in [18], which increases the risk of overlapping with excipients and impurities. In the current work, ATX retention factor was higher; thus, the analyte was eluted in a cleaner zone of the chromatogram. Regarding MPH, retention factors for the current method and [18] were quite close. The total duration of the chromatographic run was longer, but this is not an issue, considering that we determine two compounds. To sum up, we propose a procedure with similar analytical performances, more sensitive and versatile, and greener than those previously published.

3.3 | Analysis of Pharmaceutical Samples

The method was applied to solid oral dosage forms containing the studied drugs as only active component: Medikinet 10 mg modified-release hard capsules (Medice Arzneimittel Pütter GmbH & Co. KG, Iserlohn, Germany) for MPH and Strattera 10 mg hard capsule (Lilly S.A., Alcobendas, Madrid, Spain) for

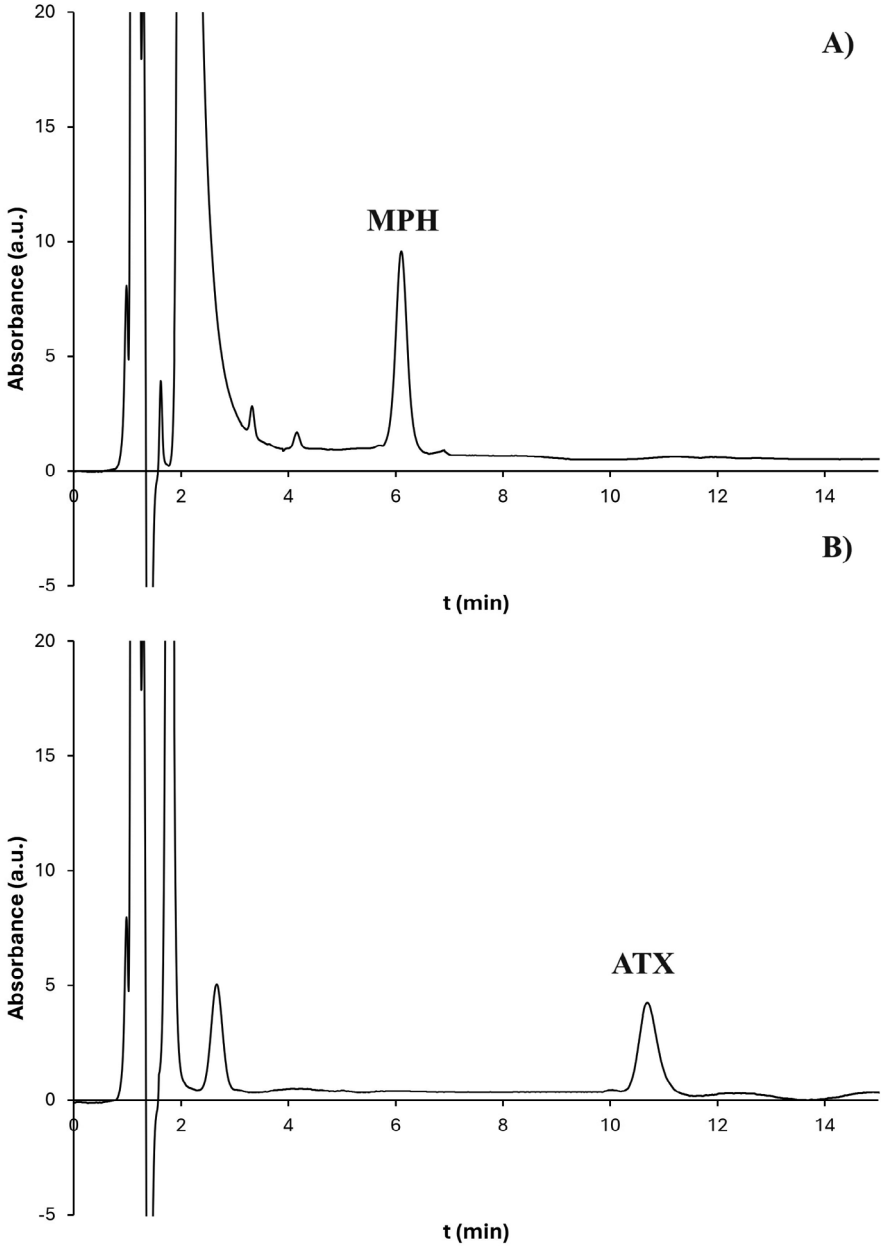

FIGURE 3 | Chromatograms obtained from the analysis of (A) Medikinet 10 mg (label claim 98.2%) and (B) Strattera 10 mg (label claim 97.3%).

ATX. The sequence run was programmed as follows: quality control (QC) 5 mg/L of both compounds, Strattera (first replicate), Medikinet (first replicate), blank standard, Medikinet (second replicate), and Strattera (second replicate) and QC 5 mg/L. The chromatograms obtained by the analysis of the samples can be seen in Figure 3A,B, respectively.

The results were as follows: 4.9 (MPH) + 5.0 (ATX), 9.8 mg (MPH), 9.7 mg (ATX), no signal, 9.8 mg (ATX), 9.8 mg (MPH), and 5.0 (MPH) + 5.1 (ATX). Therefore, there is neither drift nor bias (similar results for the replicates and QC relative recovery 9.7%–103.0%) nor cross-contamination (no signal in blank), and the label claims were inside the acceptance criteria (95.0%–105.0%).

The procedure can be carried out using generic and available reagents, laboratory material, and instrumentation. A unique solution must be prepared, for the extracting solution and the mobile phase exhibit the same composition, thus, simplifying the laboratory practice. Besides, it contains mostly unharmful chemicals and only 6.0% of toxic, volatile, and flammable organic solvent. In addition, the evaporation of 1-pentanol is hampered by its interaction with the micelles, which improves its stability. Therefore, the impact of the method on the operator health and environment is lower than hydroorganic RP-HPLC-based ones, which commonly uses a pure organic solvent to solve the pharmaceutical formulation and mobile phases containing 50%–100% methanol or acetonitrile, which are more hazardous than 1-pentanol.

Many samples can be processed simultaneously by a semiautomatic approach, and the chromatographic run only lasts 15 min. Nevertheless, a long extraction time was set to ensure a complete extraction from the pharmaceutical form. The possibility of determining both compounds using the same chromatographic

conditions is extremely useful, although they are not coprescribed in the same oral dosage form, for it allows the simultaneous analysis of MPH- and ATX-based formulations in the same workday without having to change the mobile phase. These features allow the method the analysis of many samples in a single working day.

4 | Conclusions

A procedure based on micellar liquid chromatography has been developed to quantify ATX and MPH, which are prescribed as oral dosage forms to treat ADHD. The method uses only low toxic and pollutant solutions, for the incorporation of SDS increases the solubility of the analytes, reducing the volume of organic solvent required. Besides, it is available, easy-to-handle, cost-effective, and with a high sample throughput, making it more sustainable than hydroorganic RP-HPLC. The effect of the composition of the mobile phase on the retention of the drugs was established for theoretical purpose, using a chemometric approach, and as part of the optimization of the chromatographic conditions. The method was successfully validated by the guidelines of the ICH and was proven to be enough accurate and reproducible for this sort of analysis. Therefore, it can be considered as a useful alternative for routine analysis in pharmaceutical quality control.

Author Contributions

Daniel García Ferrer: data curation, investigation, software, formal analysis, validation, writing–original draft. **Juan Peris-Vicente:** conceptualization, investigation, methodology, validation, resources, writing–original draft, project administration. **Devasish Bose:** data curation, resources, visualization, funding acquisition. **Abhilasha Durgbanshi:** conceptualization, software, writing–review and editing, supervision. **Samuel Carda-Broch:** methodology, formal analysis, writing–review and editing, visualization, supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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