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Comparative validation of amisulpride determination in pharmaceuticals by several chromatographic, electrophoretic and spectrophotometric methods

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Abstract

Nine accurate methods for determination of amisulpride in tablets: reversed phase high pressure liquid chromatography (RP-HPLC), aqueous capillary electrophoresis (CE), non-aqueous CE, normal phase (NP) and reversed-phase (RP) high performance thin layer chromatography (HPTLC) with densitometry and videodensitometry, and direct and derivative UV spectrophotometry were developed and validated. The HPLC was carried out using Nova-Pak C8 column and mobile phase consisted of acetonitrile–methanol–phosphate buffer pH 4.50 (15:5:80, v/v/v) with flow rate 1 mL min^{-1} and UV detection at 225 nm. The moclobemide was used as the internal standard. CE was performed using 75 μm × 82 cm fused silica capillary (65 cm effective), the internal standard was quetiapine. Detection was carried out at 225 nm. For aqueous analysis, the 30 mM phosphate buffer pH 6.00, 30 kV voltage and 30 ° C temperature were chosen, non-aqueous determination was performed with ammonia acetate 1 mM in acetonitrile–methanol (1:1, v/v), 30 kV voltage and 25 ° C temperature. NP-HPTLC was carried out using HPTLC silica F_{254} plates, developed with hexane–ethanol–propylamine (5:5:0.1, v/v/v) through 9 cm distance. RP-HPTLC was developed with HPTLC RP8F_{254} plates, with mobile phase of tetrahydrofuran-phosphate buffer pH 3.50 (4:6, v/v), distance 4.5 cm. Both analyses were performed in horizontal chambers and scanned with densitometer at 275 nm or videodensitometer at 254 nm. UV spectrophotometry was carried out in methanol, using 224 nm for direct assay and 258 nm (D1) for derivative assay. The precision and accuracy of all the methods were complexively compared. The highest accuracy was observed in RP-HPTLC, the highest precision was achieved in non-aqueous CE method. The differences were not significant, so all the elaborated methods can be used in routine analysis.

Keywords: Amisulpride; Analysis; Pharmaceutical formulations; High pressure liquid chromatography; Capillary electrophoresis; Thin layer chromatography; Densitometry; Videodensitometry; Spectrophotometry

1. Introduction

Amisulpride, 4-amino-*N*-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulfonyl-2-methoxy- benzamide (Fig. 1) belongs to the new generation of atypical antipsychotic drugs. Its pharmacological activity is based on the selective binding to D2 and D3 dopaminergic receptors. It has lower risk of extrapiramidal side-effects and it is relatively better tolerated than conventional antipsychotic drugs. Today amisulpride is widely used in the treatment of different kinds of schizofrenia [1].

Although there are many papers describing the determination of amisulpride in biological material: radioreceptor assay (RRA [2]), radioimmunoassay (RIA [3]), high pressure liquid chromatography (HPLC) with UV [4–7], diode array (DAD) [8], fluorescence [9–11] and mass spectrometry (MS) detection [12,13], its determination in pharmaceuticals is very sparsely described. The only paper on this subject describes the use of stationary glassy carbon electrode with cyclic, differential pulse and square-wave voltammetry for the determination of this drug in tablets and biological media (serum, urine and simulated gastric fluid samples) [14].

The analysis of pharmaceuticals is an integral and increasingly important part of an overall drug development process. Due to the increasing importance of amisulpride, a rapid and simple methods for routine analysis and quality control of commercial formulations are very desirable. There should be also mentioned, that current edition of European Pharmacopoeia contains only the classical acidimetric method for bulk material (*in substantia*) quantitation, and HPLC method for impurities identification (without quantitation).

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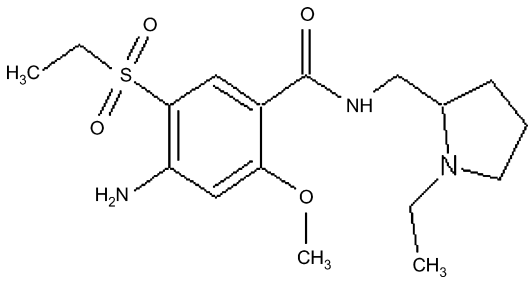

Fig. 1. The molecular structure of amisulpride.

European and United States Pharmacopoeias suggest the elaboration of new methods reducing the amount of used reagents and materials. It is related to environment pollution and harmful interference of currently used chemicals on human health. The HPTLC and CE methods are highly efficient, because several samples can be run simultaneously with very small amount of solvents in comparison to HPLC.

The aim of this work was to develop a novel, sensitive and precise set of methods for the routine quantity control of amisulpride in pharmaceutical formulations using HPLC, spectrophotometric and other, newly recommended techniques. In context of the lack of analytical methods for its determination, our proposal is a comprehensive study of the most common techniques used for this purpose—we propose nine accurate and simple analytical methods for the determination of amisulpride in commercial dosage forms.

2. Experimental

2.1. Chemicals

Amisulpride and Solian®tablets (200 mg of amisulpride, Synthelabo Groupe, Quetigny, France), moclobemide (F. Hoffman-La Roche Ltd., Basel, Switzerland) and quetiapine fumarate (AstraZeneca UK Ltd., Macclesfield, UK) were kindly supplied by manufacturers. Methanol, acetonitrile, tetrahydrofuran, hexane (all of HPLC grade) and propylamin were purchased from Merck (Darmstadt, Germany). Analytical grade anhydrous ethanol and ammonia acetate was purchased from POCH (Gliwice, Poland). The salts used to prepare phosphate buffer (KH_2PO_4 and Na_2HPO_4) were of "Ultrapure Bioreagent"® (JTBaker, UK) grade. Fresh double distilled water was used for buffer solution preparation and assays.

2.2. HPLC conditions

The Waters HPLC system (Milford, USA) consisting of Waters 515 isocratic pump, variable wavelength detector Waters 2487 at 225 nm and Rheodyne injection valve (20 μL loop) was used. The column was NovaPak C8 (4 μm, 150 mm ×3.9 mm), fed with acetonitrile–methanol–phosphate buffer pH 4.50 (15:5:80, v/v/v) with flow rate 1 mL min^{-1}. The moclobemide was used as internal standard. The sample chromatogram of the tablet extract is shown in Fig. 2.

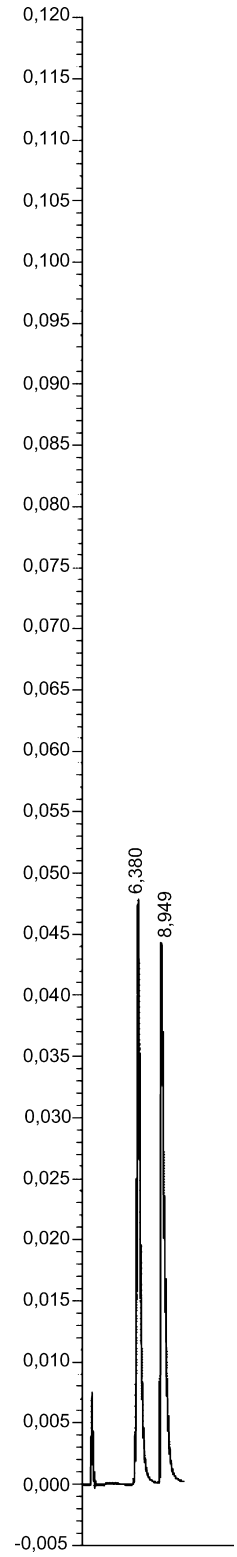

Fig. 2. The example of chromatogram of the analyzed tablets by HPLC. Peaks from left to right: amisulpride, moclobemide (IS).

2.3. CE conditions

The PrinCE CE kit with UV Lambda 1010 detector at 225 nm was used. Analysis was performed on unmodified 75 μm silica tubing of 82 cm length (65 cm to the detector). The coating on

the capillary was partially removed by burning at the point of detection, and this part was assigned onto the detection block.

During aqueous assay, capillary was conditioned by 1 M NaOH before use, and by 0.1 M NaOH (2 min, 2000 mbar pressure) before each run. The separation was performed using 1/30 M phosphate buffer of pH 6.00. The sample was spiked at 20 mbar pressure, during 3 s, at anionic end. The separation voltage was 30 kV (365.8 V cm^{-1}) and capillary was thermostated to 30 °C.

The conditioning of the capillary in non-aqueous assay was made using 1 M NaOH before use and methanol (2 min, 2000 mbar) before each run. The separation was performed in ammonia acetate 1 mM in acetonitrile-methanol (1:1, v/v) at 25 °C. The sampling and separation voltage were as in aqueous assay.

In both cases, quetiapine fumarate acted as internal standard. The sample electropherograms of the tablet extracts are shown on Figs. 3(aequous CE) and 4(non-aqueous CE) .

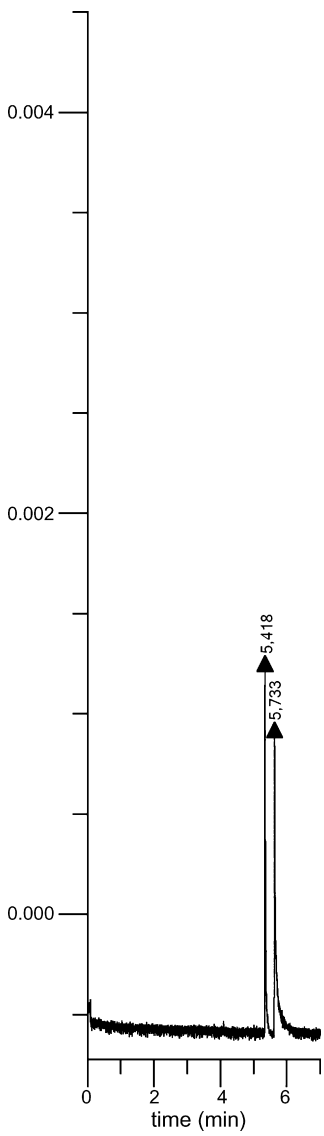

Fig. 3. The example of electropherogram of the analyzed tablets in aqueous conditions. Peaks from left to right: amisulpride, quetiapine (IS).

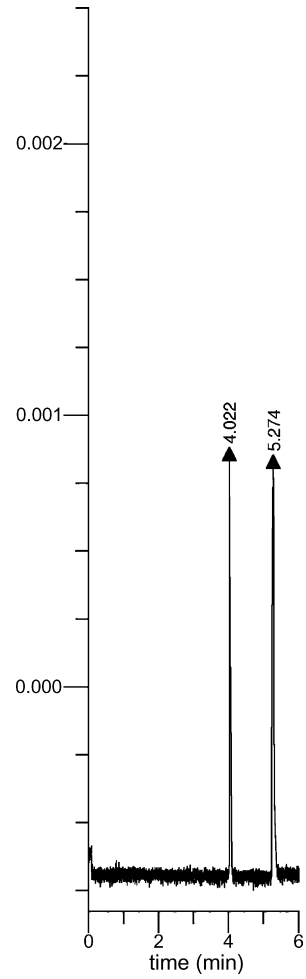

Fig. 4. The example of electropherogram of the analyzed tablets in non-aqueous conditions. Peaks from left to right: amisulpride, quetiapine (IS).

2.4. HPTLC conditions

Densitometry was carried out at 275 nm by means of Desaga CD-60 densitometer, using 0.2 × 4 mm slit. Videodensitometry was performed at 254 nm using Desaga VD-40 videoscanner with 120 s^{-1} shutter speed. The Desaga AS-30 applicator supplied with 100 μL syringe was used to apply solutions onto the plates with 10 s μL^{-1} speed.

During NP-HPTLC analysis the HPTLC silica F_{254} plates were used and developed with hexane–ethanol–propylamine (5:5:0.1, v/v/v) mobile phase on distance 9 cm in horizontal DS-II (Chromdes, Lublin, Poland) planar chromatography chambers under sandwich (unsaturated) conditions.

RP-HPTLC assay was developed in the same chambers on HPTLC RP8 F_{254} plates, with mobile phase of tetrahydrofuran-phosphate buffer pH 3.50 (4:6, v/v) on distance 4.5 cm.

The densitograms obtained during the analysis of the tablets are shown in Figs. 5(silica gel) and 6(RP8) .

2.5. UV spectrophotometric conditions

A Perkin-Elmer Lambda 15 UV double-beam spectrophotometer (Germany) was used. The methanolic solutions were

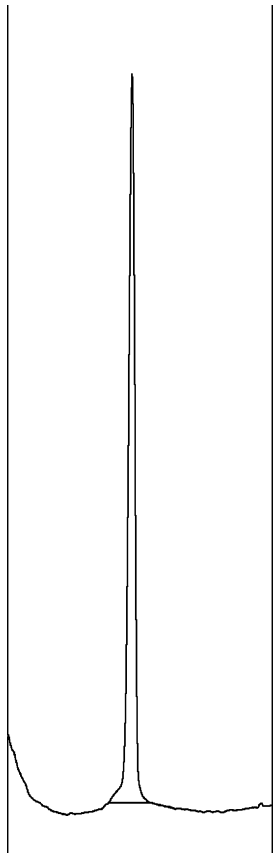

Fig. 5. The example HPTLC densitogram of the analyzed tablets on silica gel.

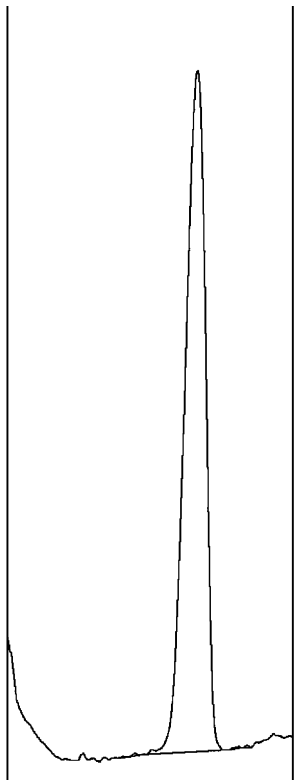

Fig. 6. The example HPTLC densitogram of the analyzed tablets on RP8.

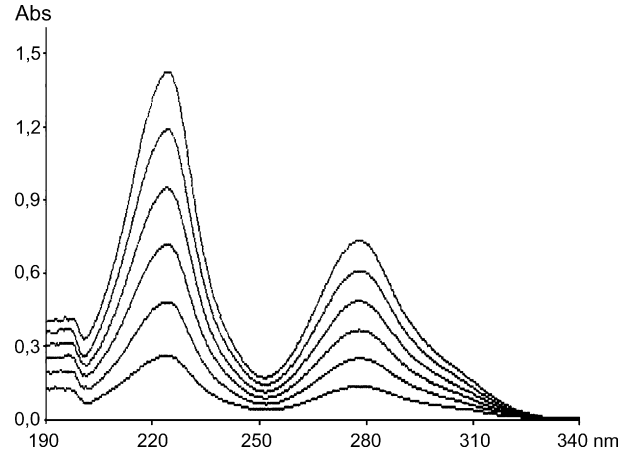

Fig. 7. The UV spectra of the amisulpride solutions in methanol (concentrations: 2–12 μg mL^{-1}).

measured in 1 cm quartz cells. The spectra were recorded using 1 mm slit and 120 nm min^{-1} scanning speed. The derivatives were calculated by built-in routines with time constant 10 s and $\Delta\lambda = 6$ nm. The assay was performed at analytical wavelength 224 nm (direct UV) and 258 nm (first derivative). In the case of derivative assay, the peak-zero technique was used. The spectra recorded for different concentrations (2–12 μg mL^{-1}) of amisulpride in methanol are shown in Figs. 7(direct spectra) and 8(first-derivative spectra)

2.6. Calibration procedure

The stock solutions of amisulpride and internal standards (1 mg mL^{-1}) were prepared in methanol. The appropriate concentrations used for calibration were obtained by dilution of stock solution, using water for aqueous CE assay and methanol for the others. The concentration range and used amounts are specified in Table 1.

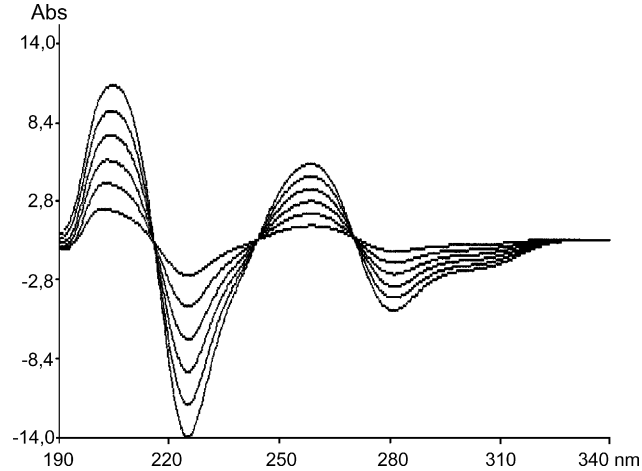

Fig. 8. The first-derivative UV spectra of the amisulpride solutions in methanol (concentrations: 2–12 μg mL^{-1}).

Table 1
The concentration range (μg mL^{-1}) and used amounts (μL) of calibration solutions of amisulpride

Method	Analyte concentration	I.S. concentration	Spotted/ injected amount
HPLC	1–20	30	20
Aqueous CE	5–50	50	[a]
Non-aqueous CE	5–50	40	[a]
NP-HPTLC	100	–	2–28
RP-HPTLC	100	–	4–34
Direct spectrophotometry	2–12	–	–
Derivative spectrophotometry	2–12	–	–

[a] Twenty millibar pressure, 3 s.

2.7. Assay procedure

The average mass of 20 Solian®tablets was determined (0.4611 g). The tablets were then ground in mortar to fine powder. The accurately weighed amounts of the powder were transferred to 25 mL volumetric flasks containing 15 mL of methanol. After adding the appropriate volume of internal standard (if needed), suspensions were mixed by reciprocating shaker for 15 min and diluted up to volume with methanol, then filtered. The obtained concentrations and amounts used in assay are given in Table 2.

3. Results and discussion

The elaboration of the methods described was begun by developing optimal conditions. We have investigated several conditions for each method and chosen optimal variants against common criteria critical in quantitative analysis, such as peak/spot shape, selectivity, total run time and linearity. In the case of HPTLC, our previous study [15] was treated as the starting point.

3.1. Validation of the calibration

In all cases, the calibration was studied by fitting obtained data to linear and quadratic ordinary least squares regression. The residuals of regression did not show significant heteroscedascity (investigated by Bartlett test), so the use of weighed regression was not necessary. The evaluation of calibration results is shown in Table 3.

Table 2
The concentration range (μg mL^{-1}) and used amounts (μL) of assay (tablet samples) solutions

Method	Analyte concentration	I.S. concentration	Spotted/ injected amount
HPLC	8.5	30	20
Aqueous CE	20	50	[a]
Non-aqueous CE	20	40	[a]
NP-HPTLC	100	–	10
RP-HPTLC	100	–	10
Direct spectrophotometry	7	–	–
Derivative spectrophotometry	7	–	–

[a] Twenty millibar pressure, 3 s.

The coefficient of determination, R^2 is most popular measure applicable to linear and polynomial regression. But this coefficient has low cognitive value in linear calibration, because high R^2 can be obtained by applying linear least squares method to significantly curvilinear data [16], when quadratic or polynomial regression is needed. So we have decided to perform the evaluation to test the linearity of calibration.

All the methods were tested for linearity by means of the Mandel's fitting test (called "a posteriori") with quadratic equation as the alternative fitting. Also the significance of quadratic term (t-value) was investigated. The densitometry on silica gel, densitometry on RP8 and videodensitometry on Si proved to be significantly curvilinear and the quadratic model was then used. In the other cases, the linearity was proven and the linear equation was sufficient. The Shapiro-Wilk test did not reject the hypothesis that residuals are normally distributed in the chosen models in all cases.

3.2. Assay in the tablets

The results of quantitation of amisulpride in tablets are presented in Table 4.

The limit of detection and limit of quantitation were obtained in HPLC and HPTLC experimentally, taking into account the signal to noise ratio. Spectrophotometric LOD and LOQ were calculated from the calibration curve estimators.

HPLC shows the best sensitivity, better than CE and spectrophotometry. The HPTLC methods cannot be simply compared with them due to using the amounts in spot as its measure. Among HPTLC methods, densitometry results with better sensitivity than videodensitometry, the best sensitivity is observed in densitometry on silica gel.

The precision of the assay was calculated as intra-day and inter-day R.S.D. ($n = 6$). The best precision was observed with non-aqueous capillary electrophoresis (inter-day 0.95%). Densitometry on silica resulted in worst precision (inter-day 3.15%). The precision of all the elaborated methods is satisfactory.

The accuracy of the results was checked by calculating the 95% confidence interval and checking if the declared amount (100% of recovery) lies inside the interval (this is exact with t-Student test for one mean). The 200 mg value lies always in confidence interval, so all the methods proved to be sufficiently accurate.

3.3. Fortified samples

In the pharmaceutical analysis, the accuracy plays very important role and should be validated in comprehensive way. The common technique used for proving the accuracy is the analysis of fortified samples (samples containing addition of the known amount of standard). The accuracy of the presented methods was additionally proved by analyzing the mixtures containing standard solutions and tablet

Table 3
Statistical evaluation of calibration: calibration curve equations, linearity and regression diagnostics

Method	Linear equation	Quadratic equation	R^2	$p(cx^2)$significance	Mandel's test	Shapiro-Wilk test
HPLC	$y = 0.1164x - 0.0073$	$y = 0.1184x - 0.0130 + 0.0001x^2$	0.99996	0.12	3.87(0.12)	0.9116(0.40)
Aqueous CE	$y = 0.0351x - 0.0117$	$y = 0.0353x - 0.0131 - 2.894 \times 10^{-6}x^2$	0.99881	0.96	0.002(0.96)	0.8644(0.20)
Non-aqueous CE	$y = 0.0298x + 0.0036$	$y = 0.0269x + 0.0297 - 2.766 \times 10^{-5}x^2$	0.99899	0.16	3.23(0.16)	0.9617(0.83)
HPTLC Si dens.	$y = 968.27x + 433.50$	$y = 1441.22x + 230.39 - 161.76x^2$	0.99543[a]	0.04	9.25(0.04)	0.9030[a](0.39)
HPTLC Si vid.	$y = 3321.71x + 988.16$	$y = 4932.98x + 296.19 - 551.13x^2$	0.99909[a]	0.006	45.8(0.0065)	0.9208[a](0.51)
HPTLC RP-8 dens.	$y = 660.14x + 230.77$	$y = 858.13x + 97.39 - 52.10x^2$	0.99967[a]	0.007	43.23(0.0071)	0.9416[a](0.67)
HPTLC RP-8 vid.	$y = 3200.73x - 494.76$	$y = 3257.23x - 532.83 - 14.86x^2$	0.99698	0.90	0.01(0.90)	0.9896(0.98)
UV direct	$y = 0.1145x + 0.0047$	$y = 0.1160x + 0.0006 - 0.0001x^2$	0.99994	0.54	0.45(0.54)	0.9614(0.83)
UV D1	$y = 0.4309x + 0.0253$	$y = 0.4362x + 0.0112 - 0.0004x^2$	0.99993	0.61	0.30(0.61)	0.9712(0.90)

Values appearing in paranthesis denotes p-values.
[a] For quadratic model.

Table 4
Statistical evaluation of results of determination of amisulpride in tablets by new nine proposed methods

Method	LOD[a]	LOQ[a]	Mean content (mg)	Recovery (%)	95% Confidence interval (mg)	Intra-day precision (%)	Inter-day precision (%)
HPLC	0.06	0.20	199.26	99.63	193.88–204.6	2.97	2.57
Aqueous CE	0.87	2.90	200.51	100.26	194.65–206.37	1.67	2.79
Non-aqueous CE	0.58	1.92	199.70	100.15	196.51–202.88	0.95	1.28
HPTLC Si dens.	0.01	0.03	200.28	100.14	193.65–206.90	3.09	3.15
HPTLC Si vid.	0.06	0.19	197.99	99.00	192.47–203.50	1.02	2.65
HPTLC RP-8 dens.	0.02	0.06	200.05	100.03	194.31–205.79	1.97	2.73
HPTLC RP-8 vid.	0.06	0.19	199.99	99.99	193.98–206.01	1.30	2.86
UV direct	0.07	0.21	197.35	98.67	193.99–200.61	1.14	1.62
UV D1	0.08	0.24	197.59	98.79	194.52–200.65	1.05	1.48

[a] $\mu g\,mL^{-1}$or μg per spot, respectively.

extracts in different fractions (50%, 100% and 150% of fortification). The results were homogenic and t-test showed no significant differences between them and the declared amount (Table 5).

3.4. Stability of solutions

The assay was also validated against the decomposition of amisulpride during the analysis. The content was quantified by densitometry on silica gel from methanolic standard solutions stored in ambient temperature and four solutions stored in stressed conditions: 60° C of temperature in methanol, 0.1 M

Table 6
Recoveries of stressed solutions of amisulpride obtained by HPTLC Si densitometric analysis

Conditions	Recovery (%) after time (h)				
	0.5	1	2	4	6
Methanol, 25 °C	98.4	97.6	97.2	99.1	96.8
Methanol, 60 °C	97.4	89.5	90.8	80.8	67.6
0.1 M HCl, 60 °C	85.8	83.1	78.3	75.4	56.4
0.1 M NaOH, 60 °C	85.7	85.3	80.5	69.0	52.51
UV 254 nm, 25 °C	47.8	32.9	4.8	1.02	0.11

Table 5
Statistical evaluation of fortified samples assay

Method	50%			100%			150%		
	Recovery	R.S.D.	Accuracy ($t(p)$)	Recovery	R.S.D.	Accuracy ($t(p)$)	Recovery	R.S.D.	Accuracy ($t(p)$)
HPLC	105.00	5.15	1.60 (0.25)	103.00	3.06	1.64 (0.24)	106.3	2.66	3.85 (0.06)
Aqueous CE	104.46	5.54	1.33 (0.31)	104.59	7.42	1.02 (0.41)	98.56	1.56	−1.62 (0.24)
Non-aqueous CE	94.91	5.16	−1.80 (0.21)	98.62	5.19	−0.46 (0.68)	95.44	4.96	−1.66 (0.23)
HPTLC Si dens.	97.80	2.34	−1.66 (0.23)	94.90	4.43	−2.10 (0.17)	93.27	7.33	−1.70 (0.23)
HPTLC Si vid.	97.80	2.78	−1.40 (0.29)	101.2	7.73	0.26 (0.81)	94.26	6.16	−1.71 (0.22)
HPTLC RP-8 dens.	103.80	5.22	1.21 (0.34)	105.60	4.74	1.93 (0.19)	103.87	4.25	1.51 (0.26)
HPTLC RP-8 vid.	95.60	4.81	−1.65 (0.23)	100.60	1.16	0.89 (0.46)	93.27	3.32	−3.76 (0.06)
UV direct	100.27	7.97	0.05 (0.95)	98.05	5.00	−0.68 (0.56)	100.56	3.61	0.26 (0.81)
UV D1	97.51	3.46	−1.27 (0.32)	98.92	0.53	−3.56 (0.07)	100.48	0.30	2.75 (0.11)

Table 7
Comparison of precision (by Bartlett and *F*-test) and accuracy (by ANOVA, Kruskal-Wallis, *t*-Student and Wilcoxon tests) of nine elaborated methods

Comparison	*F*-test		*t*-test		*U*-test	
	F	*p*	*t*	*p*	*W*	*p*
Non-aqueous CE–HPTLC Si dens.	7.56	0.04	−0.21	0.84	15	0.70
Non-aqueous CE–HPTLC RP-8 vid.	6.23	0.07	−0.12	0.91	13	0.48
Aqueous CE–non-aqueous CE	5.93	0.07	0.33	0.75	19	0.94
Non-aqueous CE–HPTLC RP-8 dens.	5.67	0.08	−0.15	0.89	17	0.94
Non-aqueous CE–HPTLC Si vid.	5.24	0.09	0.73	0.49	27	0.18
HPLC–non-aqueous CE	4.97	0.10	−0.19	0.85	21	0.70
HPTLC Si dens.–UV D1	4.66	0.12	0.95	0.37	23	0.48
HPTLC Si dens.–UV direct	3.89	0.16	1.01	0.34	23	0.48
HPTLC RP-8 vid.–UV D1	3.84	0.17	0.92	0.39	25	0.31
Aqueous CE–UV D1	3.66	0.18	1.14	0.29	24	0.39
HPTLC RP-8 dens.–UV D1	3.50	0.20	0.97	0.36	26	0.24
HPTLC Si vid.–UV D1	3.23	0.22	0.16	0.88	14	0.59
HPTLC RP-8 vid.–UV direct	3.20	0.23	0.99	0.35	25	0.31
HPHPLC–UV D1	3.06	0.24	0.69	0.51	24	0.39
Aqueous CE–UV direct	3.05	0.25	1.20	0.26	26	0.24
HPTLC RP-8 dens.–UV direct	2.91	0.27	1.04	0.33	25	0.31
HPTLC Si vid.–UV direct	2.69	0.30	0.25	0.81	17	0.94
HPHPLC–UV direct	2.55	0.33	0.77	0.46	25	0.31
Non-aqueous CE–UV direct	1.95	0.48	1.46	0.18	27	0.18
Non-aqueous CE–UV D1	1.62	0.61	1.39	0.20	26	0.24
HPLC–HPTLC Si dens.	1.52	0.66	-0.31	0.76	14	0.59
HPTLC Si vid.–HPTLC Si dens.	1.44	0.70	−0.68	0.51	16	0.82
HPTLC RP-8 dens.–HPTLC Si dens.	1.33	0.76	−0.67	0.95	17	0.94
Aqueous CE–HPTLC Si dens.	1.28	0.80	0.07	0.95	21	0.70
HPLC–HPTLC RP-8 vid.	1.25	0.81	-0.24	0.82	15	0.70
HPTLC RP-8 vid.–HPTLC Si dens.	1.21	0.84	−0.08	0.94	16	0.82
UV direct–UV D1	1.20	0.85	−0.13	0.90	16	0.82
HPLC–aqueous CE	1.19	0.85	−0.41	0.69	16	0.82
HPTLC RP-8 vid.–HPTLC Si vid.	1.19	0.85	0.63	0.54	23	0.48
HPLC–TLC RP-8 dens.	1.14	0.89	−0.26	0.80	16	0.82
Aqueous CE–HPTLC Si vid.	1.13	0.90	0.81	0.44	22	0.59
HPTLC RP-8 vid.–HPTLC RP-8 dens.	1.10	0.92	−0.02	0.99	20	0.82
HPTLC RP-8 dens.–HPTLC Si vid.	1.08	0.93	0.67	0.52	25	0.31
HPLC–HPTLC Si vid.	1.05	0.95	0.42	0.68	25	0.31
Aqueous CE–HPTLC RP-8 vid.	1.05	0.96	0.16	0.88	18	1.00
Aqueous CE–HPTLC RP-8 dens.	1.05	0.96	0.14	0.89	20	0.82
Bartlett test			$K^2 = 7.84,\ p = 0.44$			
ANOVA test			$F = 0.38,\ p = 0.92$			
Kruskal-Wallis test			$\chi^2 = 4.77,\ p = 0.78$			

NaOH and 0.1 M HCl and methanol in ambient temperature, placed in quartz cuvette under 254 nm UV lamp.

The results (Table 6) show that stressed solutions decomposed significantly during the experiment, but methanolic solution stored in ambient temperature showed no change in amisulpride content. UV irradiation is the strongest decomposing agent, 6 h of exposition results in almost total degradation of amisulpride.

The results obtained proved, that methanol was proper solvent for the amisulpride analysis. The prepared solutions were stable during analysis, and – if stored at 4 ° C – also stable during 3 months.

3.5. Pairwise comparison of precision and accuracy

The multiple comparison of precision (Bartlett test) and accuracy (ANOVA, Kruskal-Wallis test) showed no significant differences. So we decided to perform pairwise comparison of precision (by *F*-Snedecor test) and accuracy (*t*-Student or Wilcoxon, regarding to difference of precision). The results are presented in Table 7.

There are no differences in accuracy, and the precision is significantly different only between non-aqueous CE and densitometry on silica-gel (at 95% level, not at 99% level). The obtained results show that the elaborated methods are accurate and precise.

4. Conclusions

The purpose of the work presented was to validate the nine analytical methods for the quantitative determination of amisulpride in pharmaceuticals. They proved to be rapid, simple, precise and accurate. There are no differences in accuracy and precision between them, so all of them are applicable in routine

pharmaceutical control of commercially available formulations of amisulpride.

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