A Validated Time of Flight Mass Spectrometry for Quantitative Determination of Amantadine Hydrochloride and Memantine Hydrochloride

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Abstract

Introduction: Time of flight mass spectrometry was developed and validated for quantitative determination of amantadine and memantine in drug substances and products.

Materials and methods: The method was based on time of flight electron spray ionization mass spectrometry technique without preliminary chromatographic separation and made use of memantine as internal standard of amantadine, which is used as internal standard of memantine.

Results and conclusion: A linear relationship between drug concentrations and peak intensity ratios of ions of the analyzed substances is established in range of 23.80-2380.00 ng mL^{-1} for amantadine and memantine (r=> 0.998, n=6). The method is robust and reproducible with intra-and inter-assay precision (RSD % < 2.0%). The quantification limit was 23.8 ng mL^{-1} for both drugs. The described method has advantages over the reported methods as the assay was completed in less than 2 min with high accuracy and selectivity.

Introduction

Mass spectrometry (MS) is one of the most powerful analytical techniques, particularly for pharmaceutical analysis, where good selectivity and high sensitivity are often needed. The more accurate and rapid measurements, the more quickly a drug can progress towards regulatory approval. Time-of-flight mass spectrometer (TOF-MS) delivers high sensitivity, resolution, and exact mass measurements. A variety of ion source and software options makes MS a versatile choice for a range of analytical challenges [1-4].

Amantadine hydrochloride (Am), 1-aminoadamantine (Figure 1) is an antiviral agent against infection with influenza type A virus and to ameliorate symptoms when administered during the early stages of infection, as well as in management of herpes Zoster [5]. It has mild antiparkinsonism activity and thus it has been used in the management of parkinsonism, mainly in early disease stage and when the symptoms are mild. Amantadine is usually given by mouth as hydrochloride salt [6]. The analytical methods reported for analysis of amantadine HCl include, spectrophotometry, spectrofluorimetry [7-10], potentiometry [11], high performance liquid chromatography [12-14], gas chromatography [15-18], and capillary electrophoresis [19]. Memantine (Mem) is a structurally and pharmacologically related to antiviral amantadine (Figure 1). The drug is used to treat Parkinson's disease, movement disorders and dementia syndrome [20,21]. Mem acts as a non competitive inhibitor of the N-methyl-D-aspartate (NMDA) receptor complex [22,23]. Several techniques were reported for determination of Mem in a variety of matrices. These methods include, spectrophotometry, spectrofluorimetry [24-26], high performance liquid chromatography (HPLC) coupled to MS [27,28] and gas chromatography-mass spectrometry[29].

The aim of this study is to develop rapid, accurate and sensitive method for simultaneous determination of Am and Mem in drug substances and products without chromatographic separation. The recommended method was not investigated previously.

TOF ES-MS analytical technique has several advantages over the aforementioned methods, where direct HPLC and GC are unsuitable, because structures of the studied drugs lack suitable UV chromophore. There is no need for method development, a short analytical time (1.5
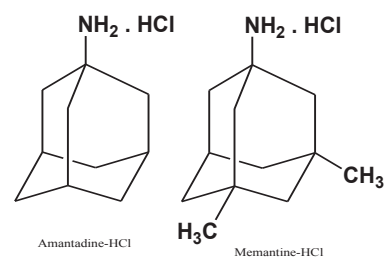

Figure 1: Chemical structures of amantadine HCl and memantine HCl.

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min), and a minimal amount of solvent being required, coupled with high sensitivity, selectivity and exact mass measurements, thereby avoiding assumptions inherent in derivatization regimes and detector response. The proposed method was validated in accordance with International Conference on Harmonization (ICH) guidelines [30].

Materials and Methods

Materials and reagents

Amantadine was purchased from Sigma Co., UK, certified to contain 99.00%, CAS No. 132112-35-7. Adamine capsule containing 100 mg amantadine per capsule (Rameda Co., Egypt) was purchased from local market. Memantine hydrochloride was kindly supplied by Adwia Co., Egypt, its purity was found to be 99.60% according to the manufacturer HPLC method [31]. Ebixa tablet containing 10.0 mg memantine hydrochloride per tablet (H. Lundbeck Co., Denmark) was purchased from the market. The following reagents and solvents were purchased and used without further purification: methanol (LC-MS grade, Fisher Scientific, UK), acetonitrile (LC-MS grade, Reidel-dehaen, UK), ultra pure water (ELGA, UK), and formic acid (Sigma-Aldrich, UK).

Apparatus and measurements

The TOF-ES-MS measurements were performed using WATERS –2795 (Waters, UK) equipped with an autosampler injector (10 μL) and Mass Lynx v 4.1. The system was operated in the following regime: electrospray voltage, 3 kv, capillary temperature, 150°C, sample solution flow rate, 0.1 mL/min. All analysis was performed in the positive ion detection mode. All samples were dissolved in a 50% solution of acetonitrile in water containing 0.1% formic acid.

Standard solutions and calibration curves

Stock solutions of Am and Mem were prepared in methanol at a concentration of 1 mg mL^{-1}, and stored at 4°C. These were further diluted with 50% aqueous acetonitrile containing 0.1% formic acid to give the appropriate working solutions. Working solutions of each drug were prepared to yield final concentrations of 23.8, 59.5, 119.0, 238.0, 595.0, 1190.0 and 2380.0 ng mL^{-1} by further dilution with the same solvent. Mem (2380.0 ng mL^{-1}) was used as internal standard for amantadine. Am (2380.0 ng mL^{-1}), was used as internal standard for memantine.

Procedure

Ten μL each of the above solutions was injected in the TOF-ES-MS under conditions mentioned above. The characteristic m/z ions used for identification and determination of Am and Mem were m/z=152 and m/z=180 [M+H]$^+$, for the two drugs respectively.

Analysis of drug products

Adamine capsule containing 100 mg amantadine per capsule (Rameda Co., Egypt) was purchased from local market. Memantine hydrochloride was kindly supplied by Adwia Co., Egypt, its purity was found to be 99.60% according to the manufacturer HPLC method [31]. Ebixa tablet containing 10.0 mg memantine hydrochloride per tablet (H. Lundbeck Co., Denmark) was purchased from the market. An accurately weight amount of powdered tablets or capsules equivalent to

about 50 mg of Am or Mem from them were transferred quantitatively to 50 mL volumetric flasks and made up to the volume with methanol. Magnetic stirrer for 30 min and filter, discard the first portion of the filtrate. The procedure was completed as mentioned above.

Calculations

The calibration curves were calculated by unweighted least-squares linear regression analysis of the concentrations of the analyte versus the peak intensity ratio of ions of analyzed substance of Am (m/z=152) to that of the IS (m/z=180). As for Mem (m/z=180) to that of IS (m/z=152) was used. The concentrations of unknown samples were determined by applying the linear regression equation of the standard curve to the unknown sample's peak intensity ratio.

Method validation

The limit of quantification of the two drugs was defined as the lowest concentration of the calibration curve.

Precision and accuracy were assessed by assaying freshly prepared solutions of the two drugs in triplicate at three concentration levels; 59.5, 119.0 and 1190.0 ng mL^{-1}. Precision is reported as relative standard deviation (RSD%) of the estimated concentrations and accuracy (Relative error%) expressed as [measured-nominal/nominal X 100].

Specificity is the ability of the method to measure the analyte response in the presence of interfering substances. For specificity determination, synthetic mixtures of different ratios of Am and Mem within the linearity range were prepared and analyzed. The recovery percent of each drug was determined.

Results and Discussion

The work includes (1) mass spectrometric identification and determinations of Am and Mem; (2) generation of the standard calibration curves; (3) quantitative analysis of Am and Mem in their drug products.

The mass spectra of Am and Mem and their internal standards are shown in Figure 2 and Figure 3. Under the conditions of TOF ES-MS in positive mode, the spectra displays intense peaks of [M + H]$^+$ with ions of the highest mass to charge, e.g. m/z=152 for Am and 180 for Mem, respectively. Linearity range was found to be in concentration range of 23.80 –2380.0 ng mL^{-1} for Am while up to 1190.0 ng mL^{-1} for Mem (Figure 4). The results of regression data were presented in Table 1.

Linear regression analysis of the data gives the equations, A=0.0034C + 0.178, r=0.9998, for Am and A=0.0029C + 0.128, r=0.998, for Mem. Where A is the peak intensity ratio for *m/z*=152/180 for Am and 180/152 for Mem, C is the concentration in ng mL^{-1} and r is correlation coefficient.

Method validation

Calibration curves for Am and Mem exhibited good linearity over the concentration range studied (23.8-2380.0 ng mL^{-1}) for Am and up to 1190.0 ng mL^{-1} for Mem in drug substances as stated in Table 1. The limit of quantification (LOQ) was chosen as the lowest calibration standard concentration (23.8 ng mL^{-1}) for the studied drugs (Table 1).

Table 2, summarizes mean values of, precision, and accuracy of intra-and inter-assay analysis. Precision and accuracy were within the

ranges acceptable for analytical and bio-analytical purposes. Intra-day precision ranged from 1.07 to 1.98% for Am while 1.03 to 2.00% for Mem in drug substances. Inter-day precision did not exceed 2.0% over the three level concentrations for three days in drug substances. The accuracy of the technique was considered satisfactory, since between-day variation over the concentration range studied was found to be less than 2%.

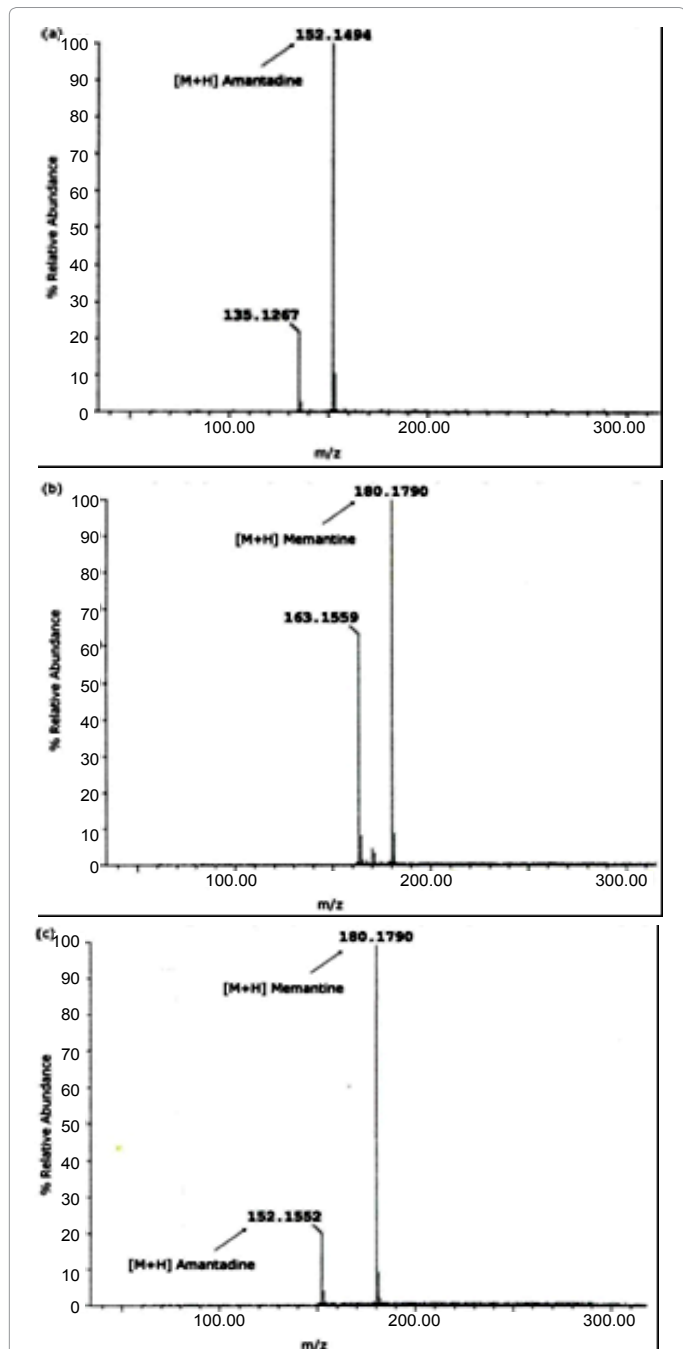The specificity was assessed by analyzing laboratory prepared

Figure 2: The typical Mass spectra of (a) amantadine, 2380.0 ng mL^{-1} (b) memantine, 2380.0 ng mL^{-1} (c) amantadine (119.0 ng mL^{-1}, analyte) and memantine (2380.0 ng mL^{-1}, internal standard) in 50% aqueous solution of acetonitrile containing 0.1% formic acid.
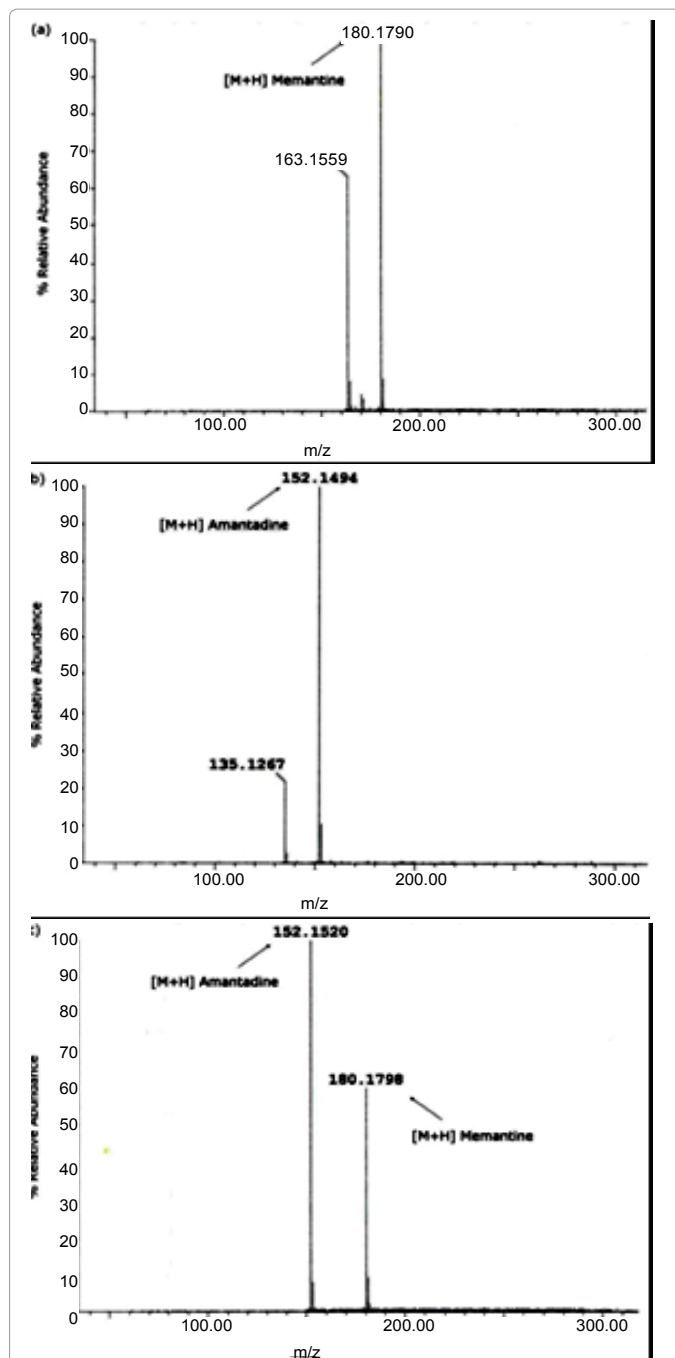

Figure 3: The mass spectra of (a)amantadine 2380.0 ng mL^{-1} (b)memantine, 1190.0 ng mL^{-1}, (C) mixture of memantine, 119.0 ng mL^{-1} (analyte) and amantadine, 2380.0 ng mL^{-1} (IS) in 50% aqueous solution of acetonitrile containing 0.1% formic acid.

mixtures of both drugs in different ratios within the linearity range. The results reveal high selectivity and sensitivity of the method (Table 3).

Application for analytical analysis of drug products

The method was applied to determine Am and Mem in Adamine capsule and Ebixa tablets respectively. The RSD% was less than 2.0%, indicating the precision of the method; the results were presented in Table 4.
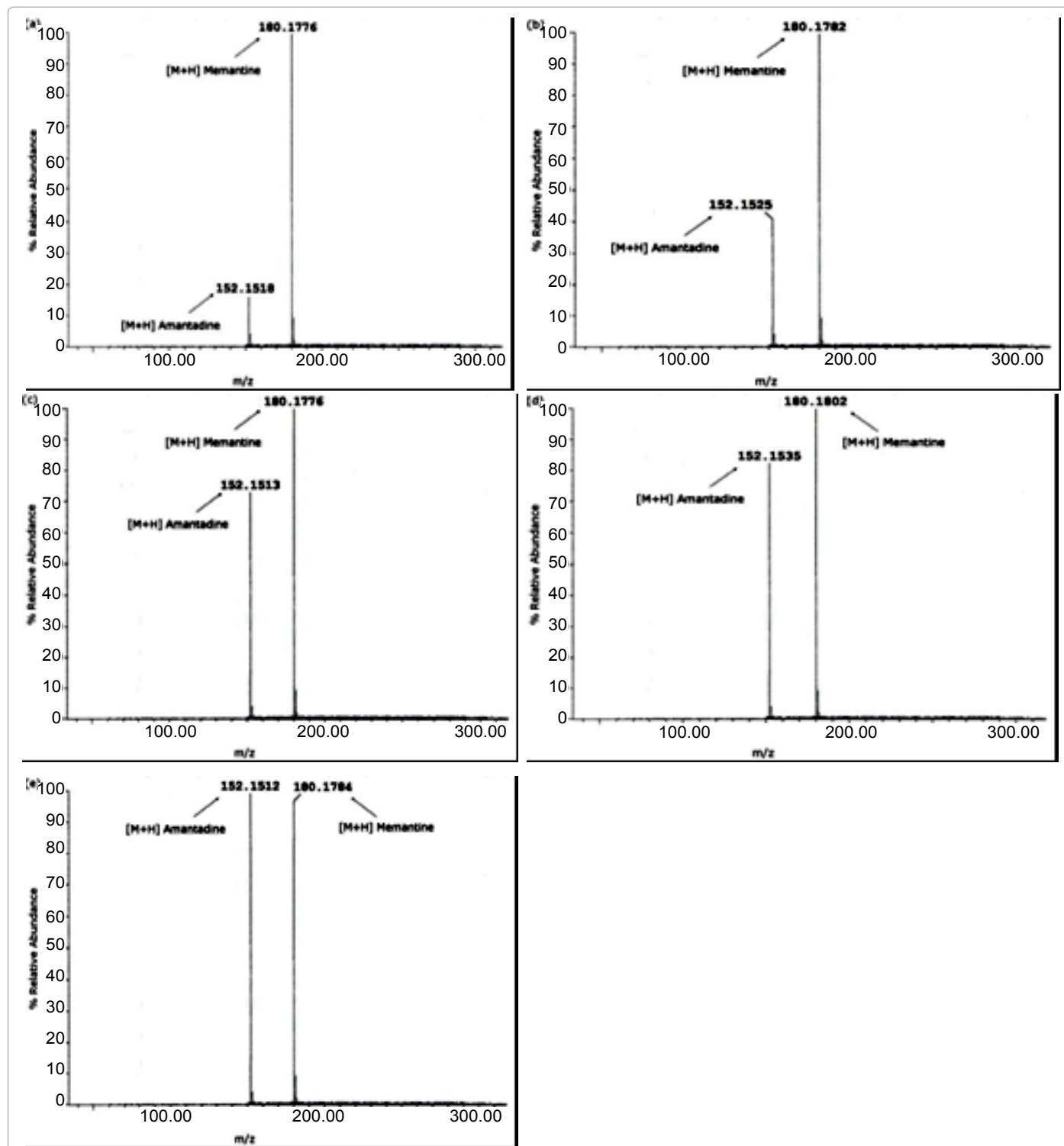
Figure 4: The mass spectra of calibration mixtures (23.8a, 59.6b, 119.0c, 1190.0d and 2380.0e ng mL^{-1})of amantadine (analyte) and memantine, 2380.0 ng mL^{-1} (IS), in 50% aqueous solution of acetonitrile containing 0.1% formic acid.

Conclusions

Analytical laboratories require accurate results, faster and more economically than ever before. This is especially true for traditional methods for rapid and non destructive analysis techniques. Therefore the present work described a newly developed TOF-MS based method for quantitative determination of Am and Mem in drug substances and drug products without chromatographic separation. The strategy of this approach consists in direct multi-ion detection of analytes with reference to internal standards with close structures to the analyte. The

Parameters	Amantadine	Memantine
Linearity ng mL^{-1}	23.80 - 2380.0	23.80 - 1190.0
LOQ ng mL^{-1}	23.80	23.80
Regression equation		
Slope (b)[a]	0.003	3.0×10^{-3}
SE of slope	2.79×10^{-5}	9.6×10^{-5}
Intercept (a)[a]	0.178	0.128
SE of intercept	0.03	0.05
Correlation coefficient (r)		
SE of estimation	0.9998	0.998
Recovery	0.060	0.097
Mean[b]± RSD%	98.83 ± 3.03	99.61 ± 3.20

[a]Regression equation, A = a + bc, where A is the peak intensity ratio for m/z = 152.0 /180.0 for Am, and A is the peak intensity ratio for m/z = 180.0/152.0 for mem, C is the concentration in ng mL^{-1}.

[b]n = 6.

Table 1: Validation report of the proposed TOF ES-MS assay for determination amantadine and memantine in drug substances.

Drug substances	Conc. ng mL^{-1}	Precision[a] RSD%		Accuracy[a] RE%	
		Intra	Inter	Intra	Inter
Amantadine	59.5	1.89	2.00	-2.67	1.59
	1190.0	1.07	1.59	-1.58	-2.19
	2380.0	1.94	1.98	-2.11	2.00
Memantine	23.8	1.03	1.96	1.14	1.97
	595.0	1.60	2.00	1.55	2.00
	1190.0	1.15	1.90	1.90	1.77

[a]n = 3

Table 2: Intra and inter-day precision and accuracy of the proposed TOF ES-MS method for analysis of amantadine and memantine in drug substances.

Ratio(ng mL^{-1})		Recovery [a] % ± RSD	
Amantadine	Memantine	Amantadine	Memantine
1	1	99.79 ± 1.07	99.04 ± 1.89
1	4	100.05 ± 2.00	100.46 ± 1.04
2	1	98.86 ± 1.66	100.18 ± 1.07

[a]Mean of five different experiments.

Table 3: Specificity of the proposed TOF ES-MS method for simultaneous analysis of amantadine and memantine in drug substances.

Preparations	TOF ES-MS	
	Recovery[a] % of claimed amount	RSD%
Adamine capsules, 100 mg mL^{-1} amantadine hydrochloride/capsule	101.55	1.45
Ebixa tablets,10.0 mg mL^{-1} memantine hydrochloride/tab	100.50	1.59

[a]Average of five different determinations

Table 4: Results of analysis of amantadine and memantine in drug products by the proposed TOF ES-MS method.

method could be routinely used for analysis of Am and Mem in drug products and biological media as well as for assessing drug purity and stability.

Acknowledgments

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