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Comparative LC–MS and HPLC analyses of selected antiepileptics and beta-blocking drugs

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Abstract

A highly sensitive and specific assay procedure based on the combination of liquid chromatography and mass spectrometry (LC–MS) has been developed for the quantitative analysis of selected antiepileptics (carbamazepine and phenytoin) and beta-blocking drugs (acebutolol, atenolol, pindolol and propranolol) using APCI as an ionization process. The measured concentration range was 100–300 ng ml^{-1} for all drugs except phenytoin (0.5–1.5 μg ml^{-1}). Analysis was based on direct injection of methanolic solutions of drugs into the mass spectrometer with the subsequent elution with a mobile phase consisting of methanol and 1% acetic acid solution (4:1) at a flow rate 1 ml min^{-1}. The mass spectrometer was programmed to permit detection and determination of either fragment or molecular ions of carbamazepine, phenytoin, acebutolol, atenolol, pindolol and propranolol at *m/e* 194.3, 252.9, 337.2, 267.1, 249.1 and 260.1, respectively. The recorded chromatograms exhibited well-resolved peaks at retention times < 1 min. The peak area was correlated linearly to the drug concentration. Intraday precision gave relative standard deviations in the range 1.75–4.02%. Compared to HPLC, the described LC–MS was faster, more sensitive and specific. Unlike HPLC, LC–MS could be applied to analyze incompletely resolved mixtures. The absolute detection limits for LC–MS and HPLC were 0.2–0.5 and 10–25 ng, respectively. Recovery studies of the investigated compounds in pharmaceutical products using LC–MS and HPLC gave mean percentages of 97.5–102.0 and 98.4–103.3, respectively. Statistical analysis of the data using *t*- and *F*-tests showed insignificant differences between both methods for the analysis of carbamazepine, phenytoin, acebutolol and atenolol in pharmaceutical formulations. However, LC–MS gave more accurate results than HPLC for determination of pindolol in tablets. Propranolol could only be determined in tablets using LC–MS.

Keywords: LC–MS; HPLC; Antiepileptics; Beta-blocking drugs; Pharmaceutical products

1. Introduction

Carbamazepine (CAZ) and phenytoin (PH) are important antiepileptics which are used clinically to control different types of seizures. Chromatographic [1–6], electrolysis-fluorimetric [7], polarographic [8] and spectrophotometric [9–11] methods have been described for the quantitative measurement of both drugs in pharmaceutical formulations and biological samples. Selective (acebutolol and atenolol) and nonselective (pindolol and propranolol) beta-blocking drugs are used extensively as antihypertensive agents. Numerous chromatographic [12–15], fluorimetric [16] spectrophotometric [17,18] and polarographic [19] techniques have been reported for the analysis of beta-blocking agents in dosage forms and biological fluids. Generally, chromatographic analysis of CAZ and PH was based on detection of the eluted compounds at shorter wavelength (~ 210 nm) [5] using UV detector, whereas, chromatographic determination of acebutolol (AC), atenolol (AT), pindolol (PN) and propranolol (PR) was based on the detection of the fluorescence intensity of the resolved drugs using the fluorescence detector [15]. Furthermore, chromatographic separations of the antiepileptics and beta-blocking drugs were influenced by the type of column and composition of the mobile phase. Improper selection of the chromatographic conditions can lead to poor detection, incomplete separation and erroneous results. Recently, the combination of HPLC and mass spectrometry (MS) applying APCI

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or ESI as an ionization process, has permitted low level quantitation of pharmaceuticals and biochemicals [20,21]. This paper reports on the application of LC–MS and HPLC techniques for the determination of CAZ, PH, AC, AT, PN and PR in laboratory-made mixtures and in pharmaceutical formulations. Comparative studies of precision, accuracy, sensitivity and selectivity criteria of LC–MS and HPLC were demonstrated.

2. Experimental

2.1. Chemicals

AC, CAZ, PH and PR were purchased from Sigma Co, USA, whereas AT and PN were supplied from RBI, USA. The authentic samples were stored at 4°C. Other chemicals and reagents were of analytical grade and the solvents were of HPLC grade.

2.2. Instruments

Liquid chromatography-mass spectrometric analyses were performed using the LC–MS system which is comprised of an LC pump (Spectra System P 2000, USA) and an MS detector (Finnigan MAT, USA) with APCI as an ionization process. The APCI conditions were: vaporization temperature, 450°C; sheath gas flow, 80 ml min^{-1}; discharge current, 5 μA; and discharge potential, 4.38 kV. The mass spectrometer was programmed to detect the positive fragment ion at 194.3 for CAZ, and the positive molecular ions at 252.9 for PH, 337.2 for AC, 267.1 for AT, 249.1 for PN and 260.1 for PR. The fragment ion of CAZ was collected using the scan parameters: scan mode MS-MS, parent ion mass 237.3, isolation width m/z 2, relative collision energy 15%. Samples were injected directly into the MS detector using a 10 μl-loop size. Elution of compounds was achieved using a mobile phase consisting of methanol and 1% acetic acid solution (4:1) at a flow rate of 1 ml min^{-1}. Analytical data were processed by the instrument build-in LCQ software.

HPLC analyses were performed using an isocratic HPLC system (Waters 2690 Separation Module, USA) equipped with photodiode array detector (PDA) and auto-sampler (Waters, USA). Chromatographic separations were performed at ambient temperature using Hypersil® BDS, C18, 5 μ, 150 × 4.6 mm column. A mobile phase composed of methanol and 1% acetic acid solution (4:1) at a flow rate of 1.5 ml min^{-1} was used. Analytical data were processed by the instrument build-in Millennium software.

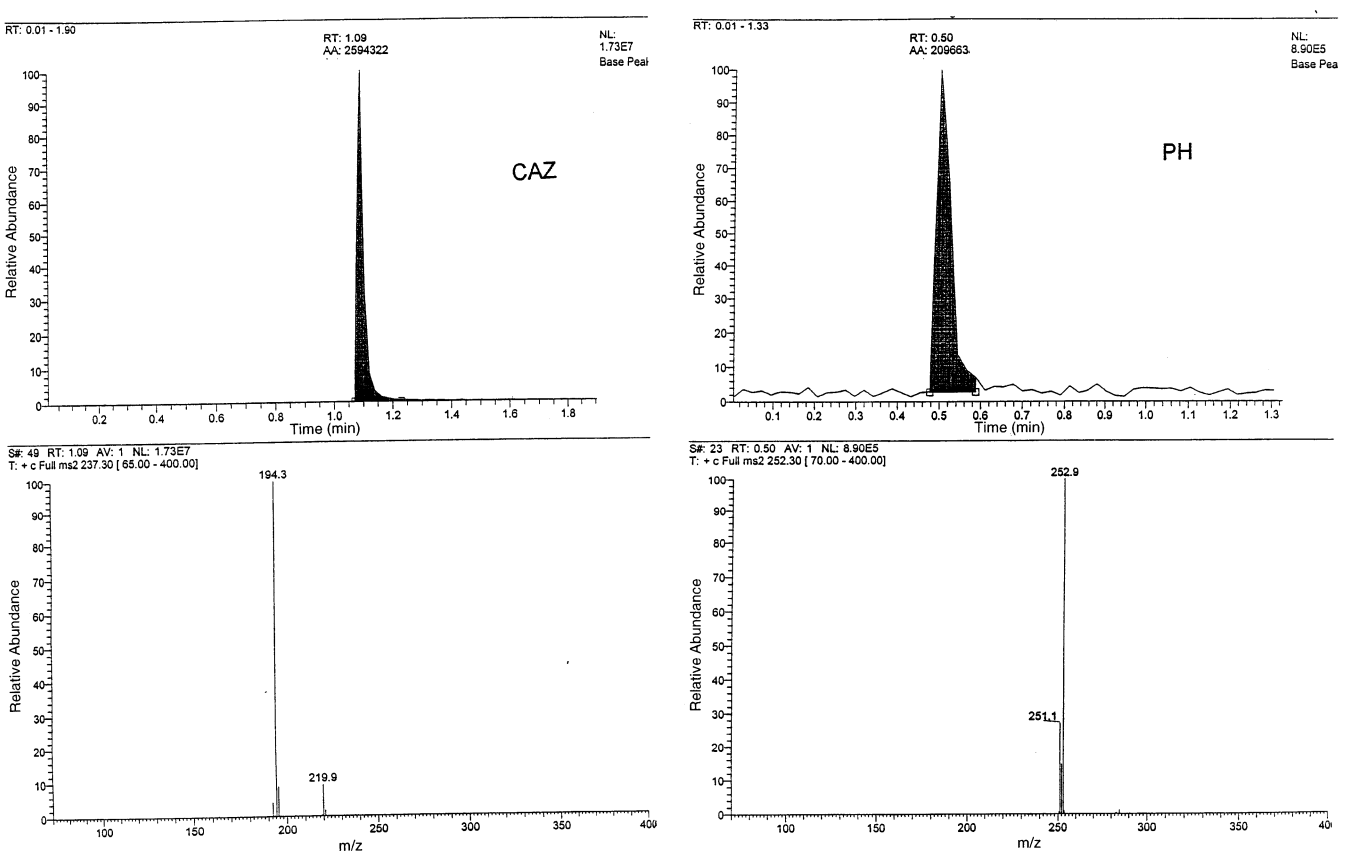

Fig. 1. LC–MS of CAZ (100 ng ml^{-1}) and PH (500 ng ml^{-1}).

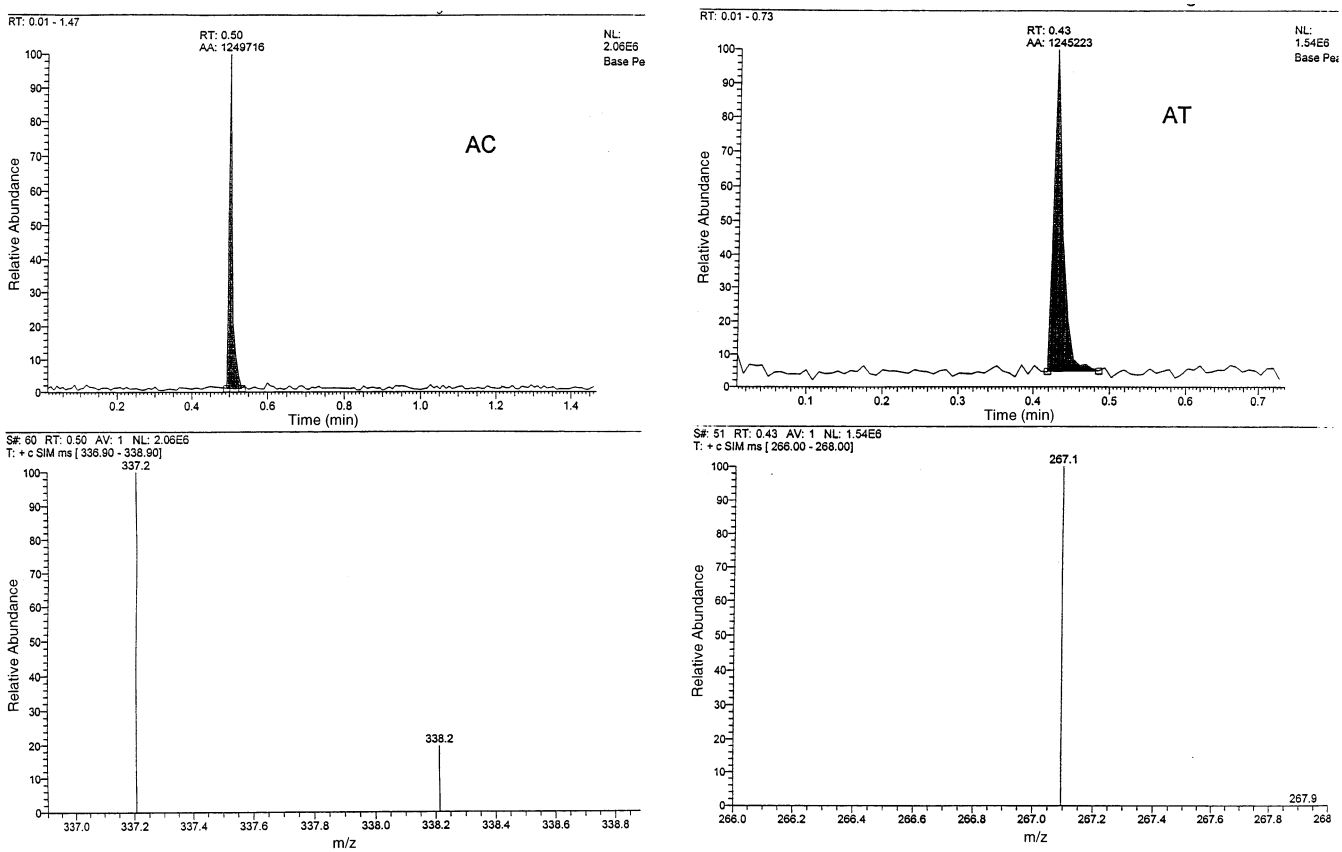

Fig. 2. LC–MS of AC (100 ng ml^{-1}) and AT (300 ng ml^{-1}).

2.3. Preparation of standards

Separate stock solutions of CAZ, PH, AC, AT, PN and PR were prepared by dissolving the appropriate weights of the authentic drugs in methanol to obtain drug concentrations of 1 mg ml^{-1}. Working standard solutions were prepared in methanol at concentrations of 10 μg ml^{-1} for LC–MS and 100 μg ml^{-1} for HPLC.

2.3.1. Calibration curves for LC–MS analyses

Separate standard solutions of CAZ, AC, AT, PN, PR were prepared by diluting appropriate volumes of the working standards with the mobile phase to concentrations of 100–300 ng ml^{-1}. For PH, standard solutions were prepared at concentration of 0.5–1.5 μg ml^{-1}. Aliquots of 10 μl were injected directly into the MS detector and the eluted compounds were monitored at the specified m/z ratios: 194.3 (CAZ), 252.9 (PH), 337.2 (AC), 267.1 (AT), 249.1 (PN) and 260.1 (PR), respectively. Peak areas were measured automatically by the instrument software. The linear regression equation relating the peak area to concentration was computed.

2.3.2. Calibration curves for HPLC analyses

Separate standard solutions were prepared by diluting appropriate volumes of the working standards with the mobile phase to concentrations of 1–3 μg ml^{-1} (CAZ, PN), 5–15 μg ml^{-1} (AC, AT) and 5–20 μg ml^{-1} (PH). A 50-μl aliquot of each solution was injected into HPLC. The linear regression equation relating the peak area to concentration was computed.

2.3.3. Intraday precision study

Samples of (CAZ, AC, AT, PN and PR) at concentrations of 250 ng ml^{-1} and 2 μg ml^{-1} (PH) were prepared in the mobile phase. A 10-μl aliquot of each solution was injected separately into LC–MS and analyzed at the previous fragment or molecular mass ions. For HPLC analysis, samples at concentrations 1.5 μg ml^{-1} (CAZ, PN) and 7.5 μg ml^{-1} (PH, AC, AT) were prepared in the mobile phase and 50 μl aliquot of each solution was injected into HPLC.

2.3.4. Selectivity of LC–MS

2.3.4.1. CAZ and PH. Laboratory-made mixtures of CAZ and PH were prepared at concentrations of 0.2–

0.5 μg ml^{-1} (CAZ) and 1–2.5 μg ml^{-1} (PH), respectively. The samples were analyzed using LC–MS by alternative measurement of mixtures at m/z 194.3 and 252.9, respectively.

2.3.4.2. AC, AT, PN and PR. Laboratory-made mixtures of AC, AT, PN and PR at concentrations of 200–500 ng ml^{-1} were prepared and analyzed using LC–MS. The mixtures were measured alternatively at m/z 337.2, 267.1, 249.1 and 260.1, respectively.

2.3.5. Analysis of commercial samples

2.3.5.1. Tablets. A single tablet, each of CAZ, AT, PN and PR, was transferred separately to 100 ml volumetric flask and mixed with 50 ml methanol. The mixture was sonicated for ~30 min and then diluted to volume with methanol. The mixture was filtered and the filtrate was passed through membrane filter to remove any particles. Appropriate aliquots were diluted and analyzed using LC–MS and HPLC procedures. The sample concentrations were determined from standard curves run simultaneously with the samples.

2.3.5.2. Capsules. The content of one capsule (AC, PH) was emptied separately in 100 ml volumetric flask and mixed with 50 ml methanol. The mixtures were sonicated for ~30 min and then diluted to volume. Appropriate aliquots of the clean filtrates were suitably diluted and analyzed as mentioned above.

3. Results and discussion

3.1. LC–MS

Recently, LC–MS has been applied progressively as an accurate, sensitive and specific analytical technique for the analysis of drugs and biochemical compounds. Unlike the classical HPLC, chromatographic detection of a drug using MS relies on monitoring the mass of the molecular or fragment ion of the drug rather than monitoring its UV absorbing properties. In this respect, determination of weak UV absorbing compounds with conventional UV detectors is always a problem, however these compounds are simply determined using mass spectrometric analysis. Moreover, due to higher specificity of LC–MS, a compound can be easily recognized by its molecular ion and any other interference from co-existing compounds can be identified, even if they co-elute. Based on these principles, it is feasible to analyze poor UV absorbing compounds, e.g. phenytoin

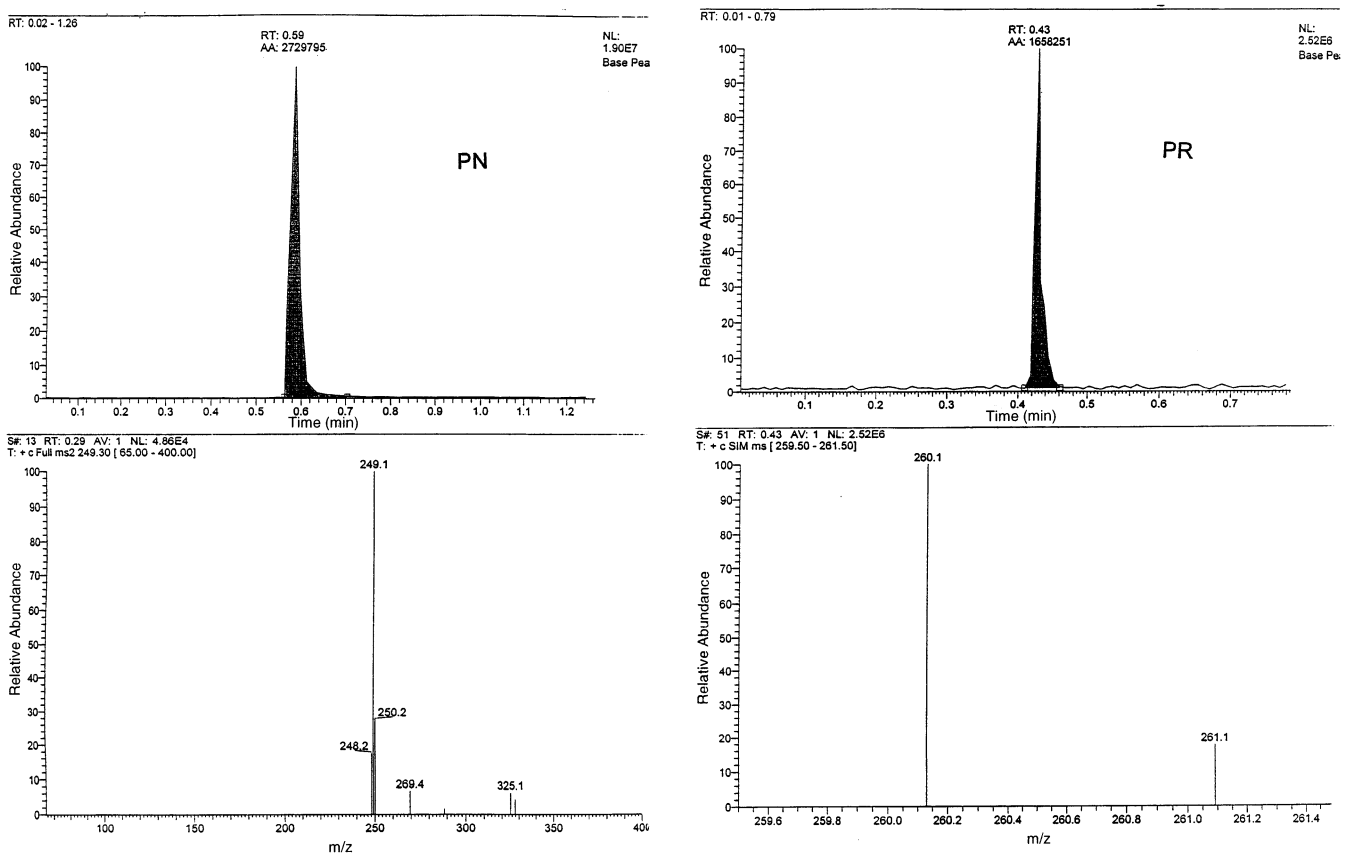

Fig. 3. LC–MS of PN (200 ng ml^{-1}) and PR (300 ng ml^{-1}).

Table 1
Calibration curve parameters for determination of antiepileptics and beta-blocking drugs by LC–MS

Comp.	Concentration range (ng ml^{-1})	Regression equation [a]	Correlation coefficient (r)
CAZ	100–300	$PA \times 10^{-6} = 0.087 + 0.025C$	0.9997
PH	500–1500	$PA \times 10^{-5} = -0.006 + 0.004C$	0.9998
AC	100–300	$PA \times 10^{-6} = -0.005 + 0.012C$	0.9996
AT	100–300	$PA \times 10^{-5} = -0.221 + 0.032C$	0.9997
PN	100–300	$PA \times 10^{-6} = 0.131 + 0.012C$	0.9934
PR	100–300	$PA \times 10^{-5} = -0.380 + 0.056C$	0.9968

[a] $PA = a + bC$ where PA, peak area; a, intercept; b, slope.

Table 2
Intraday precision for determination of CAZ, PH, AC, AT, PN and PR by LC–MS

Comp.	Actual concentration (ng ml^{-1})	Measured concentration (ng ml^{-1})	Mean $\pm$ SD	RSD (%)
CAZ	250.0	255.4	251.3 $\pm$ 10.1	4.02
		246.7		
		235.9		
		261.1		
		257.3		
PH	2000.0	2018.4	2041.1 $\pm$ 56.8	2.78
		2103.4		
		1985.7		
		1997.3		
		2100.5		
AC	250.0	257.7	255.7 $\pm$ 8.3	3.25
		250.2		
		244.6		
		265.3		
		260.8		
AT	250.0	246.8	247.3 $\pm$ 6.05	2.45
		240.7		
		256.8		
		248.3		
		243.9		
PN	250.0	245.8	251.9 $\pm$ 4.41	1.75
		255.8		
		249.7		
		251.3		
		256.5		
PR	250.0	252.5	248.8 $\pm$ 9.23	3.71
		250.9		
		240.8		
		238.6		
		261.3		

and propranolol and to quantify drug mixtures by monitoring alternatively the molecular mass ions of the individual components according to a computed scanning program, e.g. carbamazepine–phenytoin and acebutolol–atenolol–pindolol–propranolol mixtures. The facility of direct sample injection into the MS detector permits very fast detection (< 1 min) of small volumes (< 10 μl) of samples.

The positive ions of the investigated antiepileptics and beta-blocking drugs were collected firstly from the full mass spectra after direct injection of methanolic solutions of standards into the MS detector. The mass spectra of the individual compounds were scanned in the range m/z 60–400 under APCI ionization process. The protonated ions at m/z 194.3 (CAZ), 252.9 (PH), 337.2 (AC), 267.1 (AT), 249.1 (PN) and 260.1 (PR) were selected to quantify the examined drugs in laboratory-made mixtures and pharmaceutical formulations. In order to obtain maximum sensitivity and specificity, CAZ was collected as a fragment ion at m/z 194.3, whereas PH, AC, AT, PN and PR were collected as parent (molecular) ions. Single ion monitoring (SIM) scan mode was used to increase the method selectivity, particularly, when mixtures of antiepileptics or beta-blocking drugs were analyzed. Typical LC–MS chromatograms of the individual compounds were shown in

Table 3
Determination of CAZ and PH in laboratory-made mixtures using LC–MS

Mixture	Concentration found		Recovery (%)	
CAZ (ng ml^{-1})+PH (μg ml^{-1})	CAZ	PH	CAZ	PH
200.0+1.0	199.1	0.97	99.6	97.1
300.0+1.5	315.3	1.44	105.1	96.0
400.0+2.0	411.8	2.08	102.8	104.0
500.0+2.5	520.8	2.66	104.6	104.4
Mean			103.0	100.4
±SD			2.5	4.4

Figs. 1–3. As indicated, the chromatograms displayed well-resolved peaks with almost steady and stable baseline at retention times less than 1 min. Quantitation of the examined compounds using LC–MS was based on peak area measurement which was calculated automatically by the build-in LCQ software. Calibration curve parameters (Table 1) for the examined compounds indicated linear correlations in the concentration range 100–300 ng ml^{-1} for CAZ, AC, AT, PN and PR and 0.5–1.5 μg ml^{-1} for PH. The correlation coefficients were in the range 0.9934–0.9998. The absolute detection limit range for CAZ, PH, AC, AT, PN and PR was 0.2–0.5 ng. Intraday precision of LC–MS using control samples gave relative standard deviations of 1.75–4.02% (Table 2). To indicate the specificity of LC–MS, laboratory-made mixtures of CAZ–PH and AC–AT–PN–PR were prepared and analyzed. Negligible interference from co-existing compounds in the analysis of individuals was observed. The data proved excellent specificity of LC–MS method (Tables 3 and 4). The accuracy of LC–MS was assessed by determining the recovery percentages of the individual drugs in their pharmaceutical products. Mean recovery percentages of 99.8 (CAZ), 102.0 (PH), 97.5 (AC), 99.4 (AT), 100.5 (PN) and 97.8 (PR) were computed.

3.2. HPLC analysis

HPLC separations of the examined antiepileptics and beta-blocking drugs were performed at ambient temperature using Hypersil® BDS, C18 column and a mobile phase consisting of methanol–1% acetic acid solution (4:1). The resolved compounds were detected using a photodiode array detector at 258 nm. A base deactivated silica (BDS) C18 column was found to be more reliable than the untreated ODS column for analysis of CAZ, PH, AC, AT and PN, as well-defined and good resolved peaks were recorded. Under the selected chromatographic conditions, PH, CAZ appeared at 4.04 and 4.59 min (Fig. 4), respectively, whereas, PN, AT and AC appeared at 2.21, 2.87 and 4.15 min (Fig. 5), respectively. Under the selected chromatographic conditions, the developed HPLC method failed to identify PR due to poor detection of the compound by UV detector. Calibration curves were linear over the concentration range 1–3 μg ml^{-1} (CAZ, PN), 5–15 μg ml^{-1} (AC, AT) and 5–20 μg ml^{-1} (PH). The correlation coefficient was in the range 0.9913–0.9999 (Table 5). The absolute detection limits of CAZ, PH, AC, AT and PN were 10–25 ng. The precision of the HPLC method was evaluated by replicate analysis of drug samples at concentrations of 1.5 μg ml^{-1} (CAZ and PN) and 7.5 μg ml^{-1} (PH, AC, AT). Relative standard deviations of 1.71–3.82% were computed (Table 6). HPLC analyses of control laboratory-made mixtures of CAZ–PH and AC–AT–PN gave average percentages of 95.0–99.9% (Tables 7 and 8). Recovery studies of the examined drugs in pharmaceutical formulations gave mean percentages of 98.4 (CAZ), 103.3 (PH), 98.9 (AC), 98.5 (AT) and 99.8 (PN) (Table 9). Statistical analysis of the data by application of *t*-test and *F*-test indicated an insignificant difference between LC–MS and HPLC procedures for determination of CAZ, PH, AC and AT. In the case of pindolol, LC–MS gave more accurate values than HPLC as a significant difference ($F = 14.6$) between both methods was reported. This may be attributed to a low-dose of pindolol in

Table 4
Determination of AC, AT, PN and PR in laboratory-made mixtures using LC–MS

Mixture (ng ml^{-1})	Concentration found				Recovery (%)			
AC+AT+PN+PR	AC	AT	PN	PR	AC	AT	PN	PR
200 [a]	206.3	191.2	204.9	208.6	103.2	95.6	102.5	104.3
300	301.3	308.3	295.8	287.4	100.4	102.8	98.6	95.8
400	396.3	378.4	423.3	369.5	99.1	94.6	105.8	92.4
500	526.3	489.7	516.6	507.4	105.3	97.9	103.3	101.5
Mean					102.0	97.7	102.6	98.5
±SD					2.79	3.67	2.99	5.39

[a] Concentration of each component in mixture.

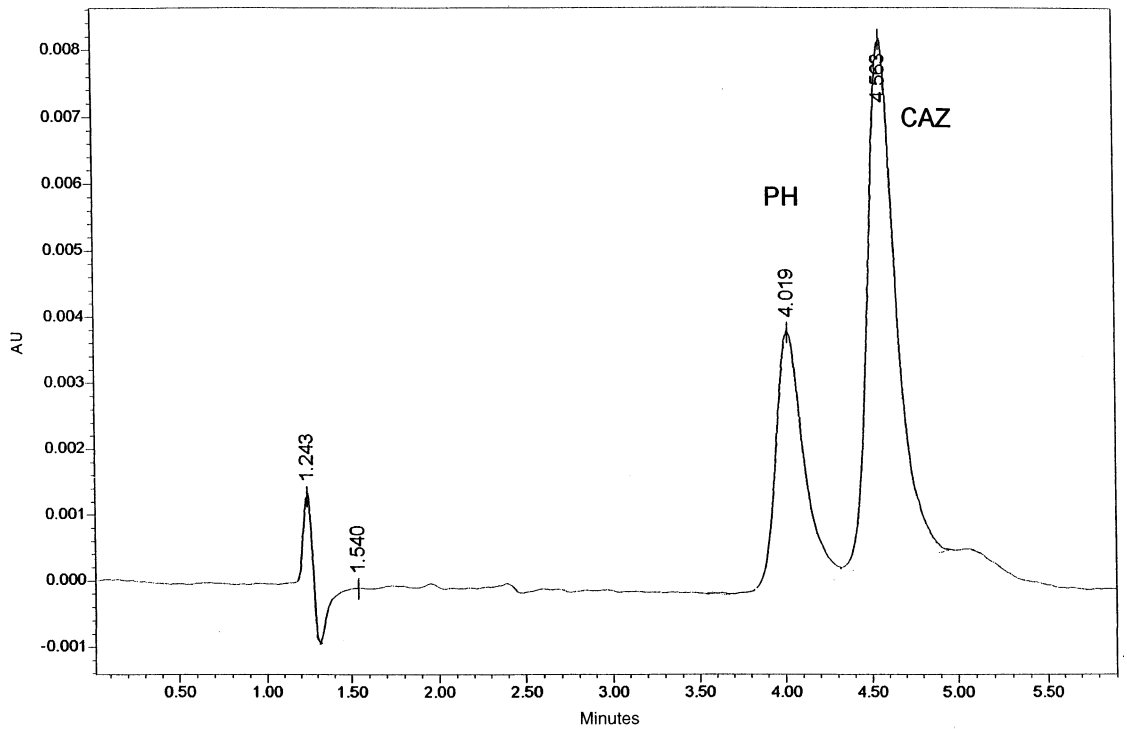

Fig. 4. HPLC chromatogram of PH (5 μg ml^{-1}) and CAZ (1 μg ml^{-1}). Stationary phase: BDS, C18. Mobile phase: methanol:1% acetic acid solution (4:1).

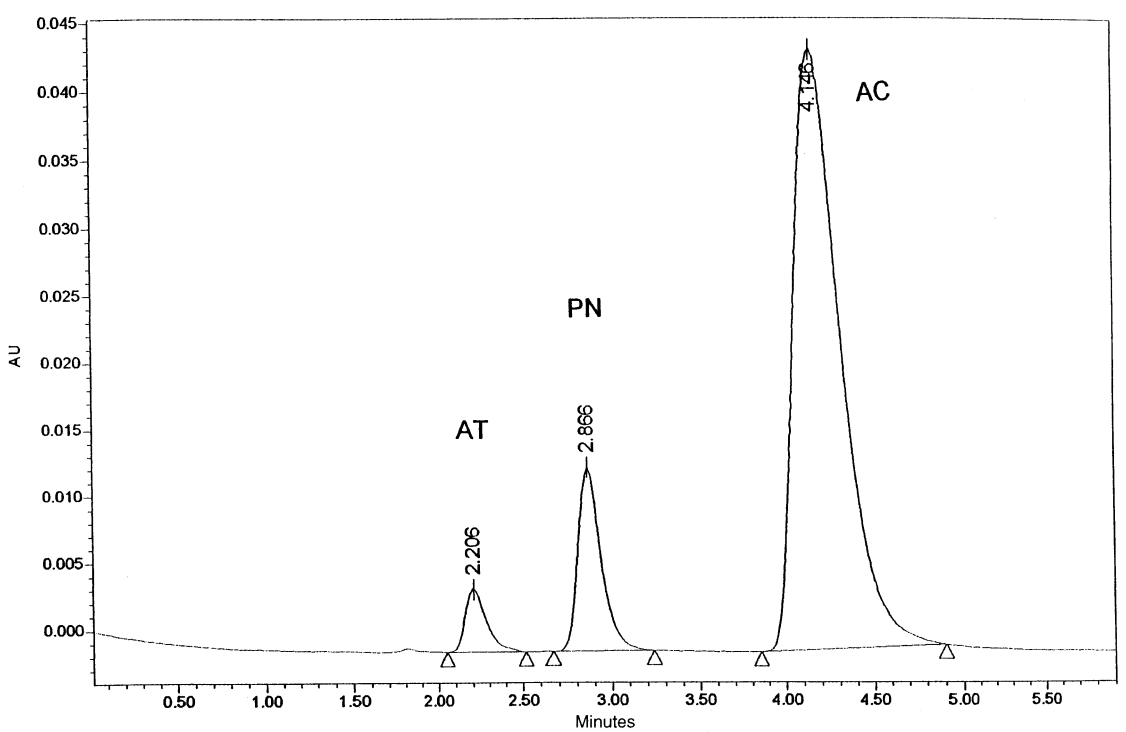

Fig. 5. HPLC chromatogram of AT (7.5 μg ml^{-1}), PN (2 μg ml^{-1}) and AC (7.5 μg ml^{-1}). Stationary phase: BDS, C18. Mobile phase: methanol:1% acetic acid solution (4:1).

tablets. On the other hand, propranolol could be determined in tablets at low-dose using LC–MS, however, HPLC failed to detect propranolol at these levels due to weak UV absorption properties of the drug.

Table 5
Calibration curve parameters for determination of antiepileptics and beta-blocking drugs by HPLC

Comp.	Concentration range (μg ml^{-1})	Regression equation [a]	Correlation coefficient (r)
CAZ	1–3	$PA \times 10^{-4} = 0.255 + 4.988C$	0.9999
PH	5–20	$PA \times 10^{-4} = -0.029 + 0.322C$	0.9966
AC	5–15	$PA \times 10^{-5} = -0.441 + 1.148C$	0.9913
AT	5–15	$PA \times 10^{-4} = -0.101 + 0.518C$	0.9977
PN	1–3	$PA \times 10^{-4} = 0.071 + 6.330C$	0.9998

[a] $PA = a + bC$ where PA, peak area; a, intercept; b, slope.

Table 6
Intraday precision for the determination of CAZ, PH, AC, AT, PN by HPLC

Comp.	Actual concentration (μg ml^{-1})	Measured concentration (μg ml^{-1})	Mean ± SD	RSD (%)
CAZ	1.5	1.48 1.61 1.55 1.63 1.60	1.57 ± 0.06	3.82
PH	7.5	7.79 7.66 7.55 7.46 7.51	7.59 ± 0.13	1.71
AC	7.5	7.11 7.34 7.55 7.60 7.58	7.44 ± 0.21	2.82
AT	7.5	7.69 7.40 7.38 7.60 7.71	7.56 ± 0.16	2.11
PN	1.5	1.46 1.42 1.53 1.47 1.45	1.46 ± 0.04	2.74

4. Conclusions

The developed LC–MS and HPLC techniques have been used successfully in the quantitative analysis of CAZ, PH, AC, AT, PN and PR in tablets and capsules. Although, LC–MS and HPLC are of comparable accuracy and precision, LC–MS has the advantages of very short time of analysis (< 1 min), higher sensitivity, selectivity and feasibility of direct injection of samples into MS detector. Furthermore, LC–MS is very useful in the analysis of drugs with poor UV absorbing properties such as propranolol and phenytoin and to quantify drugs at low-doses such as pindolol and propranolol. Finally, unlike HPLC, LC–MS is capable of determining mixtures of antiepileptics or beta-blocking drugs, even when they were not eluted completely.

Table 7
Determination of CAZ and PH in laboratory-made mixtures using HPLC

Mixture (μg ml^{-1})	Concentration found		Recovery (%)	
CAZ + PH	CAZ	PH	CAZ	PH
1.0 + 5.0	0.91	4.89	91.0	97.8
1.5 + 7.5	1.43	7.34	95.3	97.9
2.0 + 10.0	1.91	9.82	95.5	98.2
2.5 + 12.5	2.47	11.85	98.8	94.8
Mean			95.1	97.2
± SD			3.2	1.6

Table 8
Determination of AC, AT and PN in laboratory-made mixtures using HPLC

Mixture (μg ml^{-1})	Concentration found			Recovery (%)		
AC+AT+PN	AC	AT	PN	AC	AT	PN
10.0+10.0+2.0	10.1	10.3	1.9	101.0	103.0	95.0
5.0+5.0+2.0	5.2	5.1	2.0	104.0	102.0	100.0
15.0+15.0+2.0	14.8	14.1	1.8	98.7	94.0	90.0
20.0+20.0+2.0	19.2	19.8	1.9	96.0	99.0	95.0
Mean				99.9	99.5	95.0
±SD				3.4	4.0	4.1

Table 9
Determination of carbamazepine, phenytoin, acebutolol, atenolol, pindolol and propranolol in commercial samples

Comp.	Recovery (%) [a]		
	LC–MS		HPLC
Carbamazepine [c]	99.8 ± 2.1		98.4 ± 3.3
		t-value [b]: 0.80	
		F-value [b]: 2.47	
Phenytoin [d]	102.0 ± 1.1		103.3 ± 2.0
		t-value: 0.91	
		F-value: 3.31	
Acebutolol [e]	97.5 ± 1.6		98.9 ± 3.5
		t-value: 0.81	
		F-value: 4.79	
Atenolol [f]	99.4 ± 1.8		98.5 ± 1.9
		t-value: 0.53	
		F-value: 1.11	
Pindolol [g]	100.5 ± 1.1		99.8 ± 4.2
		t-value: 0.25	
		F-value: 14.6	
Propranolol [h]	97.8 ± 2.2		

[a] Mean of five determinations ± SD.
[b] Theoretical *t*- and *F*- values at *P* 0.05 are 2.31 and 5.19, respectively.
[c] Tegretol®, labeled to contain 200 mg of carbamazepine per tablet, Novartis, Switzerland.
[d] Epanutin®, labeled to contain 100 mg phenytoin per capsule, Parke-Davis, Germany.
[e] Sectral®, labeled to contain 100 mg acebutolol per capsule, Rhone-Poulenc-Rorer, France.
[f] Tenormin®, labeled to contain 50 mg atenolol per tablet, Zeneca, UK.
[g] Visken®, labeled to contain 5 mg pidolol per tablet, Novartis, Switzerland.
[h] Inderal®, labeled to contain 10 mg propranolol per tablet, Zeneca, UK.

Acknowledgements

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References

[1] R. Panchagnula, K. Kaur, I. Singh, C.L. Kaul, Determination of carbamazepine in plasma, urine and formulations by high-performance liquid chromatography, Pharm. Pharmacol. Commun. 4 (1998) 401–406.
[2] A.K. Hanada, V.P. Shedbalkar, H.L. Bhalla, Stability-indicating HPLC method for carbamazepine, Indian Drugs 33 (1996) 559–562.
[3] D. Chollet, E. Castella, P. Combe, V. Amera, High-speed liquid-chromatographic method for the monitoring of carbamazepine and its active metabolite, carbamazepine 10,11-epoxide, in human plasma, J. Chromatogr. B Biomed. Appl. 683 (1996) 237–243.
[4] J. Hallbach, H. Vogel, W. Guder, Determination of lamotrigine, carbamazepine and carbamazepine epoxide in human serum by gas chromatography-mass spectrometry, Eur. J. Clin. Chem. Clin. Biochem. 35 (1997) 755–759.
[5] M.M. Bhatti, G.D. Hanson, L. Schultz, Simultaneous determination of phenytoin, carbamazepine and 10,11-carbamazepine epoxide in human plasma by high-performance liquid chromatography with ultraviolet detection, J. Pharm. Biomed. Anal. 16 (1998) 1233–1240.
[6] H.L. Huang, D.S. Zhi, X.D. Zhou, Advanced analysis of phenytoin sodium and phenobarbital sodium in blood using high-performance capillary chromatography, Fenxi Ceshi Xuebao 18 (1999) 45–52.
[7] Z.T. Pan, L.F. Yao, Electrolysis — fluorimetric determination of carbamazepine in tablets, Fenxi-Huaxue 26 (1998) 997–1000.
[8] Z. Zhang, S. Chen, W. Huang, F. Xu, Polarographic determination of carbamazepine in the presence of sodium dodecanesulfonate, Yaoxue-Xuebau 28 (1993) 312–316.
[9] C. Goicoechea, A. Olivieri, Simultaneous determination of phenobarbital and phenytoin in tablet preparations by multivariate spectrophotometric calibration, Talanta 47 (1998) 103–108.
[10] G.A. Saleh, Charge-transfer complexes of barbiturates and phenytoin, Talanta 46 (1998) 111–121.
[11] M. Korany, M. Bedair, S. Haggag, Extraction-spectrophotometric determination of phenytoin in capsules and plasma using potassium permanganate/dicyclohexano 24-crown 8, Talanta 46 (1998) 9–14.
[12] Y.P. Patel, S. Patil, I.C. Bhoir, M. Sundaresan, Isocratic, simultaneous reversed phase high-performance liquid chromatography estimation of six drugs for combined hypertension therapy, J. Chromatogr. A 828 (1998) 283–286.
[13] A.M. El-Walily, Analysis of nifedipine–acebutolol hydrochloride binary combination in tablets using UV derivative spectroscopy and capillary gas chromatography and high-performance liquid chromatography, J. Pharm. Biomed. Anal. 16 (1997) 21–30.

[14] M.S. Leloux, W.M. Niessen, R.M. Van-der-hoeven, Thermospray liquid chromatography-mass spectrometry of polar beta blocking drugs: preliminary results, Biol. Mass Spectrom. 20 (1991) 647–649.
[15] M. Abdel-Hamid, High-performance liquid chromatographic determination of propranolol and 4-hydroxypropranolol in serum, J. Clin. Pharm. Ther. 13 (1988) 183–189.
[16] T.P. Ruiz, C. Martnez-Lozano, V. Tomas, J. Carpena, Simultaneous determination of propranolol and pindolol by synchronous spectrofluorimetry, Talanta 45 (1998) 969–976.
[17] I. Panderi, M. Parissi-Poulou, Simultaneous determination of clopamide–pindolol combination in tablets by zero-crossing derivative spectrophotometry, J. Pharm. Biomed. Anal. 12 (1994) 151–156.
[18] C.V.N. Prasad, C. Parithar, K. Sunil, P. Parimoo, Simultaneous determination of amiloride hydrochloride, hydrochlorothiazide and atenolol in combines formulations by derivative spectroscopy, J. Pharm. Biomed. Anal. 17 (1998) 877–884.
[19] Z. Nasierowska, J. Deres, J. Suffczynski, W. Stanczak, Polarographic determination of acebutolol, Farm. Pol. 40 (1984) 87–89.
[20] Y. Li, K. Neufeld, J. Chastain, A. Curtis, P. Velagaleti, Sensetive determination of erythromycin in human plasma by LC–MS/MS, J. Pharm. Biomed. Anal. 16 (1998) 961–970.
[21] M.D. Moyer, T. Johannsen, R.J. Stubbs, Determination of 2-chlorodeoxyadenosine (cladribine, 2-CDA) in human plasma by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, J. Pharm. Biomed. Anal. 17 (1998) 45–51.