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Simultaneous HPLC-determination of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol in multi-component combinations

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ABSTRACT

A RP-HPLC method for quantification of analgin (ANA), caffeine (CAF), domperidone (DOM), ergotamine tartarate (ERGTAR) and paracetamol (PAR), singley or admixed in multi-component pharmaceutical preparations, was developed, optimized and validated. The analyzed drug substances could be elegantly separated on a reversed phase column[Nucleosil C_{18} (10μm, 15cm×4.6mm, *i.d.*)] isocratically by using a mixture of sodium dihydrogen *o*-phosphate (0.02M) - methanol (30:70, v/v) as the mobile phase with UV-detection at 240 nm. Significant linearity was observed in the ranges of 54-600μg mL^{-1}(ANA), 18-180μg mL^{-1} (CAF), 10-900μg mL^{-1} (DOM), 1-45μg mL^{-1} (ERG.TAR) and 30-300μg mL^{-1} (PAR). The challenge of the developed method is its suitability for the successful separation and quantification of each of the named drug substances; either in their laboratory-prepared mixtures and/or in the complex matrices of pharmaceutical preparations containing them in single run. Statistical evaluation of the results was obtained by adopting the proposed method and those of official ones has been undertaken by applying the student *t*-testing, *F*-ratio calculation and by one-way ANOVA assessment.

KEYWORDS

Analgin;
Caffeine;
Domperidone;
Ergotamine;
Paracetamol;
RP-HPLC.

INTRODUCTION

Analgin and paracetamol are commonly prescribed analgesics, while caffeine is a central nervous stimulant, domperidone is a specific dopamine blocker usually recommended as an antiemetic. Ergotamine is a semi-synthetic dopamine D_2-agonist usually prescribed as anti-migrainic drug (Figure 1)[2]. Medicines containing different combinations of them are normally intaken for the relief of severe migrain headache[1].

Chromatography is a widely adopted methodology for the analysis and stability investigations of most drugs in pharmaceutical formulations and in quite similar complex matrices. Different chromatographic procedures, such as high-performance liquid chromatography (HPLC)[3-7] and/or thin-layer chromatographic (TLC) fractionation coupled with densitometric scanning[8,9] have been suggested for the determination of

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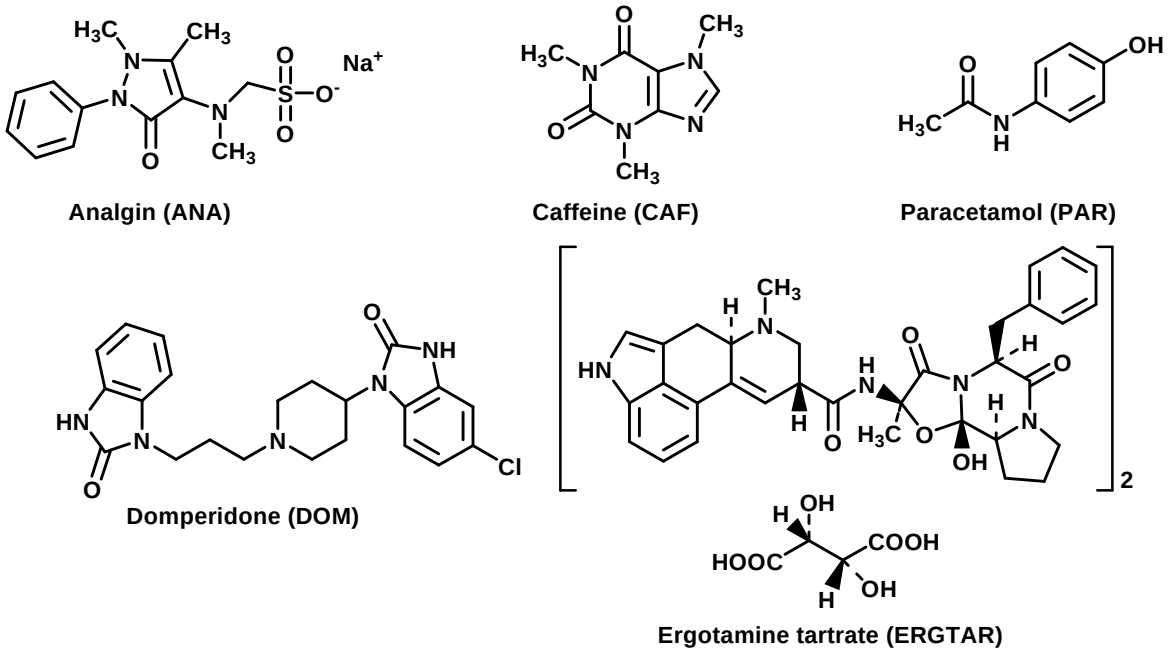

Figure 1 : Chemical structures of the five components

analgin in different pharmaceutical preparations.

Different chromatographic methods, such as HPLC-analysis[10-13], or gas chromatography (GC)[14] have been suggested for the determination of caffeine and paracetamol separately or combined in some pharmaceutical preparations. Several analytical methods, like HPLC[15-19] and/or coupled chromatography/densitometry[20,21] are described for the quantification of domperidone in various pharmaceutical formulations.

Several HPLC methods[22-25] have been suggested for the determination of ergotamine in some dosage forms.

However, all of the cited methods don't include a procedure for simultaneous determination of all the named five drug substances in their multi-component mixtures, especially in cases of complex matrices, like dosage formulations. In modern analytical laboratory, there is always a need for significant method for analyzing such multi-component dosage forms.

The main aim of this work was to develop a simple and fast but accurate analytical method for quantifying analgin, caffeine, domperidone, ergotamine tartarate and paracetamol simultaneously in multi-component dosage formulations containing most or even all of them. In a link-frame of cooperation between pharmaceutical industry and universities, such a target could be achieved with affording great time and effort-saving through the complete analysis of all the named substances in two different pharmaceutical preparations (tablets) by adopting the optimized and validated methodology. Satisfactory short complete analysis-time (~8 minutes) could be achieved by following the described experimental conditions.

EXPERIMENTAL

Chemicals and reagents

HPLC-grade methanol, Sodium dihydrogen *o*-phosphate and tartaric acid.

Samples

Pure reference samples

All reference substances were kindely supplied by R&D-unit at ADCO, Cairo-Egypt: Analgin, Zhejiang Haisen Pharm. Co. Ltd., Dongyang city, Zhejiang-China, BNo.:2007-06050, 100.39±0.81% pure, according to the BP-2008 method (volumetry)[2]. Caffeine, Sinochem Ningbo Chem. Co. Ltd., Ningbo City, Zheijiang-China, BNo.:200705177, its purity was 99.25±0.96%, as assayed by the BP-2008 method (titrimetry)[2]. Domperidone, Dr.Reddy's Pharm. Co., Greenlands, Hyderabad-India, BNo.:Dplm 049D06, purity 100.02±0.79%, as assayed by the BP-2008 method (HPLC)[2]. Ergotamine tartrate, Biesterfeld International

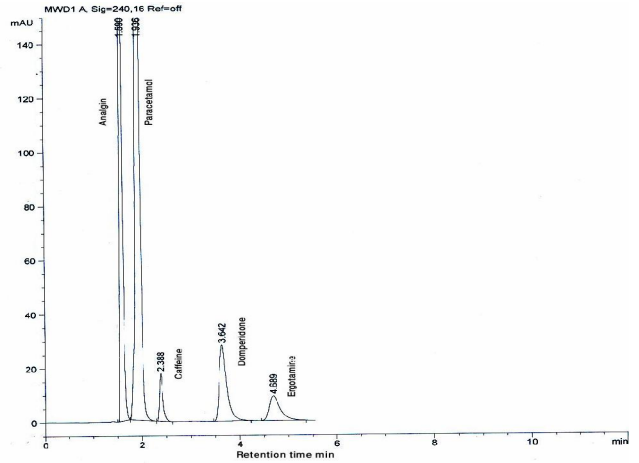

Figure 2 : Liquid chromatographic separation of analgin (1.59 min.), paracetamol (1.94 min.), caffeine (2.39 min), domperidone (3.64 min.) and ergotamine tartarate (4.69 min.) containing 600µg mL^{-1}, 50µg mL^{-1}, 10µg mL^{-1}, 2µg mL^{-1} and 250µg mL^{-1} of analgin, caffeine, domperidone, ergotamine tartrate and paracetamol respectively in methanol (by following the specified chromatographic conditions)

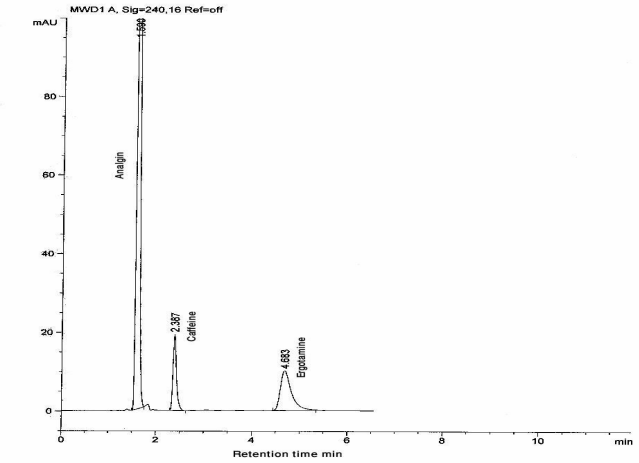

Figure 4 : Liquid chromatographic separation of analgin (1.59 min.), caffeine (2.389 min.) and ergotamine tartarate (4.68 min.) from Amigrain™ tablets containing 600µg mL^{-1}, 50µg mL^{-1} and 2µg mL^{-1} for analgin, caffeine and ergotamine tartrate respectively in methanol (by following the specified chromatographic conditions)

GmbH, Hamburg-Germany, BNo.:2007-62133, 99.43±0.37 %, was assessed by the BP-2008 method (spectrophotometry)[2]. Paracetamol, 99.86±0.87 % pure, as determined by the method described in the BP-2008 (HPLC)[2].

Market dosage formulations

Two multi-component market samples, namely, sample-1 (Amigrain™ tablets) and sample-2 (No-migrain® tablets) were collected randomely from local

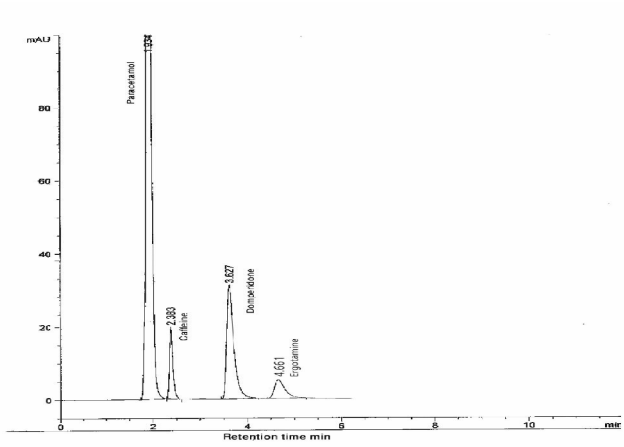

Figure 3 : Liquid chromatographic separation of paracetamol (1.93 min.), caffeine (2.38 min.), domperidone (3.63 min.) and ergotamine tartarate (4.66 min.) from No-migrain® tablets containing 50µg mL^{-1}, 10µg mL^{-1}, 1µg mL^{-1} and 250µg mL^{-1} for caffeine, domperidone, ergotamine tartrate and paracetamol respectively in methanol (by following the specified chromatographic conditions)

pharmacies in Cairo-Egypt. Amigrain™ tablets, manufactured by Arab Drug Co.(ADCO), Cairo-Egypt, BNo.:810173, labelled to contain 1mg ergotamine tartarate, 300mg analgin & 25mg caffeine in each tablet. Each No-migrain® tablet, manufactured by Amoun Co., Cairo-Egypt, B.No:1613 was claimed to contain 1mg ergotamine tartarate, 50mg caffeine, 10mg domperidone & 250mg paracetamol.

Stock standard solutions

- Standard solutions were stable for at least a week on keeping refrigerated (~5°C).
- Standard stock methanolic solutions of each drug substances;
- Analgin stock standard solution (1.8mg mL^{-1})
- Caffeine stock standard solution (0.6mg mL^{-1})
- Domperidone stock standard solution (3mg mL^{-1})
- Ergotamine tartarate stock standard solution (0.2mg mL^{-1})
- Paracetamol stock standard solution (1.5mg mL^{-1})

and their mixtures were prepared by careful complete dissolution of accurately weighed aliquots of the substance(s) in calculated volumes of methanol.

Apparatus and experimental conditions

Liquid chromatograph consisted of an isocratic pump, a variable wavelength UV-detector, equipped with autosampler injector and integrator (Model 1100

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Series, Agilent USA), Stationary phase: Nucleosil C_{18} analytical column (10µm ,15cm×4.6mm, *i.d.*), Alltech (USA). Mobile phase composed of 20mM NaH_2PO_4 solution and CH_3OH (30:70, v/v (pH5.3±0.2)) was running isocratically at 1.5mL min^{-1}. The mobile phase was filtered through a 0.45-µm millipore membrane and was degassed for about 15 minutes in an ultrasonic bath prior to use. The rate of flow was controlled at 1.5mL min^{-1}, isocratically at ambient temperature (~25°C) with UV-detection at 240nm. The samples were filtered also through a 0.45-µm membrane filter.

Calibration

Aliquot volumes of analgin (1.8mg mL^{-1}), caffeine (0.6mg mL^{-1}), domperidone (3mg mL^{-1}), ergotamine tartarate (0.2mg mL^{-1}) and paracetamol (1.5mg mL^{-1}) stock solutions were accurately transfered separately into a series of 100-mL volumetric flasks, and the content of each flask was completed to volume with methanol to cover the concentration ranges of 54-600µg mL^{-1} (ANA), 18-180µg mL^{-1} (CAF), 10-900µg mL^{-1} (DOM), 1-45µg mL^{-1} (ERGTAR) and 30-300µg mL^{-1} (PAR). The samples were then chromatographed by considering the following chromatographic conditions: Stationary phase; a C_{18}-Nucleosil column (10µm, 15cm×4.6mm, *i.d*) from Altech Associates, Inc.(Deefield, Il-USA), mobile phase NaH_2PO_4-solution (20mM) -methanol (30:70,v/v), filtered and ultrasonicated prior to use. Sample volumes each of 5µL were injected in replicates. To reach good equilibria, the analysis was usually performed not before passing ~50-60mL of the mobile phase, just for conditioning and pre-washing of the stationary phase. The relative peak area values were plotted *versus* their corresponding concentrations to get the calibration graphs and to compute the corresponding regression equations. Concentrations of unknown samples of ANA, CAF, DOM, ERGTAR and PAR were determined by using the obtained regression equation.

TABLE 1 : System suitability parameters

Parameters[a]	Separated compounds				
	ANA	CAF	DOM	ERGTAR	PAR
Retention time t_R	1.59	2.388	3.642	4.689	1.936
Retention factor *K*	1.12	2.19	3.85	5.25	1.59
Resolution R_s	3.88		6.17	3.47	2.97
Selectivity factor α	1.23		1.48	1.32	1.18
Tailing factor	1.22	1.116	1.26	1.29	1.197
Theoretical plate (column efficiency)	5002	6315	3130	2254	5017

[a]Reference values[26,27], R_s > 1.5, T = 1, for a typical symmetrical peak α > 1, *K* =1 - 10 are acceptable, Theoretical plate =The higher the value, the more the column efficiency

Analysis of laboratory prepared mixtures

Laboratory prepared mixtures containing different ratios of ANA, CAF, DOM, ERGTAR and PAR were prepared, as detailed in TABLE 2, and the mixtures were chromatographed as under the calibration curves starting from: "5µL were injected…". The concentration of each component was calculated from its corresponding regression equation.

Analysis of pharmaceutical dosage forms

Twenty tablets were weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to one tablet was accurately weighed and transferred into a 100mL volumetric flask. The mixture was shaked with 1mL 1% (w/v) aqueous tartaric acid solution and 50mL of methanol (to achieve complete dissolution of ergotamine tartrate). The solution was subjected to ultrasonic bath for 10 min and the volume was completed with methanol and filtered through filter paper. Further dilution was made to obtain the proper concentrations using methanol as diluting solvent then chromatographed as described under the construction of calibration curves starting from: "5µL were injected…". The concentration of each component was calculated from its corresponding regression equation.

RESULTS AND DISCUSSION

The literature does not contain any HPLC methods for the simultaneous assay of ANA, CAF, DOM, ERGTAR & PAR in mixtures. In the present work, a simple, accurate, and sensitive HPLC method for the simultaneous determination of them has been developed, validated, and applied for the quantitation of these five drug substances in pharmaceutical dosage forms.

Method optimization

Choice of stationary phase

Different types of stationary phase C_8 and C_{18} col-

umns with different dimensions and particles size were tried (Agilent C_8 Zorbax, Agilent C_{18} Zorbax, Agilent C_8 Eclipse and Agilent C_{18} Eclipse columns), to get the best stationary-mobile phase match. It was clearly found that Nucleosil[(ODS),10µm (15cm×4.6mm, *i.d.*)] gave the most suitable resolution for quantification of all the named five drug substances.

Choice of mobile phase

Different mobile phases at different pH values and varying organic modifiers including acetonitrile and methanol have been tested for optimizing the HPLC-separation. The mobile phase selection was based on peak parameters (symmetry, tailing), run time, ease of preparation and cost. The optimum mobile phase composition, with a final pH of 5.3 [±0.2], was found to be sodium dihydrogen o-phosphate (20mM) in bidistilled water - methanol, in the ratio of 30:70, by volumes Flowing at rate of 1.5mL min^{-1} was found to be quite satisfactory for the good resolution and determination of all the studied drug substances, singley and/or admixed. Increasing the ratio of sodium dihydrogen *o*-phosphate or decreasing the flow rate leads to delay in the elution of all peaks, also decrease in ratio of sodium dihydrogen o-phosphate or increase in flow rate leads to bad resolution between all peaks.

Choice of detector wavelength

For determination of the optimum HPLC-UV detector wavelength, the method was repeated using the same chromatographic conditions at different wavelengths (220-300nm), where, the optimum wavelength with ideal sensitivity and low noise was at 240nm and is quite far from the cut-off of water and methanol.

Upon applying the optimum chromatographic con-

TABLE 2 : Analysis of laboratory prepared mixtures of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol by the proposed HPLC method

Ratio[a]					Recovery (%)[b]				
ANA	CAF	DOM	ERG TAR	PAR	ANA	CAF	DOM	ERGTAR	PAR
300	25	-	1	-	98.89	99.77	-	99.22	-
150	200	-	2	-	100.11	98.70	-	99.75	-
150	50	-	4	-	99.64	102.00	-	99.50	-
-	50	10	1	250	-	99.77	100.56	99.22	98.43
-	25	20	2	125	-	97.80	100.27	100.26	100.31
-	15	10	0.5	50	-	101.49	99.18	97.82	102.00

[a]Different postulated ratios of analgin , caffeine, domperidone, ergotamine tartarate and paracetamol, respectively.
[b]Average of 3 experiments

TABLE 3 : Summary of the validation parameters of the proposed HPLC method for the determination of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol

Parameter	ANA	CAF	DOM	ERGTAR	PAR
Linearity					
Slope	0.0055	0.017	0.0341	0.0335	0.0066
Intercept	-0.0019	-0.0105	-0.0176	-0.0024	+0.0096
Correlation coefficient (*r*)	0.9999	0.9998	0.9999	0.9998	0.9999
Range (µgmL^{-1})	54-600	18-180	10-900	1-45	30-300
Accuracy : Mean ±RSD (%)	100.97±0.736	99.49±0.840	99.86±0.640	99.82±0.212	99.87±0.488
Precision (RSD%)					
Repeatability[a]	0.126-0.071	0.282-0.176	0.524-0.473	0.387-0.192	0.619-0.535
Intermediate precision[b]	0.552-0.471	1.473-1.016	1.34-0.982	0.684-0.132	0.853-0.712
Limit of detection (µgmL^{-1})	10.20	5.44	1.61	0.207	6.00
Limit of quantitation (µgmL^{-1})	30.91	16.47	4.88	0.627	18.18

[a]The intraday (n=6), average of six concentrations repeated three times within the day.
[b]The interday (n=6), average of six concentrations repeated three times in three successive days

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dition, well resolved sharp peaks of ANA, CAF, DOM, ERGTAR & PAR, appeared at retention times of ~1.59, 2.39, 3.64, 4.69 and 1.94 minutes in order .Only very little practical deviations from the mean t_R-values of the resolved drugs were observed, but not in the same days. The total run time for a complete quantification of all the five drug substances was ~8 minutes. Figure 2 shows a typical chromatogram obtained from the analysis of a laboratory prepared mixture of reference ANA, PAR, CAF, DOM & ERGTAR, in order, by using the proposed method. Figures 3 & 4 show typical chromatograms obtained from the analysis of the two commercial multi-component mixtures Amigrain™ and Nomigrain® tablets using the proposed method.

System suitability

System suitability parameters[26,27] calculated under the optimized experimental conditions. These five components could be eluted in forms of symmetrical peaks quite away from each other and the retention time values of the separated peaks together with other chromatographic parameters are collected in TABLE 1. The TABLE describes the calculated resolution values (R_s) as well as selectivity factor (α) which ensures complete or 100% separation of the components under investigation. The Tailing factor of each drug peak also revealed linear isotherm peak elution without tailing.

Method validation

Range and linearity

Linear relationships were obtained between relative peak areas and concentrations for ANA, CAF, DOM, ERGTAR& PAR in concentration range of 54-600μg mL^{-1}, 18-180μg mL^{-1}, 10-900μg mL^{-1}, 1-45μg mL^{-1} and 30-300μg mL^{-1}, respectively. The regression equations were computed from the relative peak area of each drug substance(peak area of drug to that of external standard (180μg mL^{-1}, 60μg mL^{-1}, 300μg mL^{-1}, 30μg mL^{-1} and 150μg mL^{-1} for ANA, CAF, DOM, ERGTAR and PAR in order) *versus* their corresponding concentrations (TABLE 3).

Limit of detection and limit of quantification

For each standard, The limit of detection (LOD) was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte could be reliably detected, at a signal-to-noise (S/N) ratio. Determination

TABLE 4 : Comparison between the results of the analysis of the studied drug substances in two tablets formulations by the proposed HPLC-method and the official(BP-2008) methods

Dosage forms	The Proposed HPLC-Method					Official method[2]				
	PAR	ERGTAR	DOM	CAF	ANA	PAR[a]	ERGTAR[c]	DOM[a]	CAF[b]	ANA[b]
Sample-1 tablets	-	104.38±0.607	-	103.00±0.488	99.71±0.313	-	106.80±0.790	-	108.61±1.720	108.10±0.131
Sample-2 tablets	99.30±0.174	101.00±0.575	99.81±0.251	95.60±0.526	-	99.70±0.406	104.30±1.510	99.00±0.349	99.80±0.464	-
SAT[d] (Mean±RSD %)	99.00±0.848	98.92±1.326	100.65±1.874	99.40±1.228	100.25±1.236	-	-	-	-	-

[a]HPLC-analysis, [b]Titritimetry (volumetry), [c]UV-Analysis, [d]Standard addition technique
(all results are avarage of five experiments)

TABLE 5 : Statistical comparison of the results obtained by the proposed HPLC-method for determination of pure samples of analgin, caffeine, domperidone, ergotamine tartarate and paracetamol with the results of the official(BP-2008) methods

Parameter	The proposed HPLC method					Official method[2]				
	ANA	CAF	DOM	ERGTAR	PAR	ANA[a]	CAF[b]	DOM[c]	ERGTAR[b]	PAR[a]
Mean	100.97	99.49	99.86	99.82	99.87	100.39	99.25	100.00	99.43	99.86
Concentration range(μg mL^{-1})	54-600	18-180	10-900	1-45	30-300	-	-	-	-	-
SD	0.743	0.836	0.639	0.211	0.487	0.813	0.955	0.786	0.373	0.867
RSD (%)	0.736	0.840	0.640	0.212	0.488	0.809	0.962	0.786	0.375	0.868
Variance	0.552	0.699	0.408	0.045	0.237	0.661	0.912	0.618	0.139	0.752
F-value (5.005)[d] n=6	1.197	1.305	1.515	3.124	3.173	-	-	-	-	-
Student's t-test(2.228)[d] n = 6	1.153	1.415	0.303	0.199	0.022	-	-	-	-	-

[a]Titration (volumetry), [b]Potentiometric titration, [c]UV-method, [d]Figures in parentheses represent the corresponding tabulated values of *t* and *F* at p=0.05

of the S/N ratio was performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples. An S/N ratio of 3:1 is generally considered acceptable for estimating the detection limit.

Limit of quantitation (LOQ) was determined by establishing the minimum concentration at which the analyte can be reliably quantitated. An S/N ratio of 10:1 is generally considered acceptable for estimating the quantition limit.

According to ICH[28,29] recommendations the approach based on SD-values of the responses and the corresponding slopes, the detection and quantitation limits were calculated. The theoretical values were assessed practically as they are given in TABLE 2.

Accuracy

To study the accuracy of the proposed method, laboratory prepared mixtures containing various amounts of ANA, CAF, DOM, ERGTAR & PAR were prepared and analyzed by the proposed method. The mean percentage recovery and SD were calculated from the recovery experiment and compared with official methods for the same compounds in similar pharmaceutical preparations. Results are presented in TABLE 2.

Precision

The precision of the proposed method, expressed as RSD%, was determined by analysis of 3 different concentrations within the linearity range for each ingredient. The intraday precision was assessed from the results of 6 replicate analyses of same concentration on a single day. The interday precision was determined from the same concentration analyzed on 3 consecutive days. The results of intraday and interday precision are illustrated in TABLE 3.

Specificity

For testing the specificity of the method, the percentage recovery of each component was determined in mixture with possible interfering materials, excipients. In application of the proposed methods to pharmaceutical formulation no interference from the tablet's excipients appeared. Hence the proposed method is able to determine the named drugs selectively in their pharmaceutical formulations. Standard addition technique (SAT) has been also applied to assess the accuracy and specificity of the proposed method, as shown in TABLE 4.

Robustness

The robustness of a method is its ability to remain unaffected by small changes in parameters. Several modified chromatographic conditions,small changes in proportions of different components, by up to ±0.5 % mainly of the organic part of the mobile phase, in addition to the ionic strength of the o-phosphate salt component ,flow rate , pH of the mobile phase (5.3±0.2) and different production lot number of Nucleosil C_{18} column, were applied which did not affect the good separation of the five components.

Stability

Analyzing commercial samples kept at room temperatures (~22±0.5°C) on the laboratory bench or in the refrigerator (~5°C) for two weeks has been carried out which resulted in RSD% values within 1.0%.

Statistical analysis[30,31]

Statistical evaluation of the results obtained by applying the proposed method and those of the Official (BP-2008) ones has been undertaken by the student *t*-testing, *F*-ratio calculation and by one-way ANOVA assessment,where it was concluded that there is no statistically significant differences between them (TABLE 5).

CONCLUSION

The proposed HPLC method is simple, and the total run time for the chromatographic run is less than 8 minuites for the 5 components of Amigrain™ tablets No-migrain® tablets. The quantitation of each component was not affected by any of the possible interfering substances included in tablet manufacturing. The method is accurate and precise, as is evident from the results of the recovery study and the low RSD% values. It can be concluded that the proposed HPLC method has great promise for the routine determination of cited drugs single, combined in laboratory prepared mixtures and in the pharmaceutical preparations.

Full Paper

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