

Rapid and Simultaneous Determination of Acetylsalicylic Acid, Paracetamol, and Their Degradation and Toxic Impurity Products by HPLC in Pharmaceutical Dosage Forms

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Aims: Determinations of drug impurity and drug degradation products are very important from both pharmacological and toxicological perspectives. Establishment of monitoring methods for impurities and degradation products during pharmaceutical development is necessary because of their potential toxicity. The aim of this study was to develop a rapid and simultaneous determination method for paracetamol and acetylsalicylic acid (ACA) and their degradation and toxic impurity products by high performance liquid chromatography (HPLC) in pharmaceutical dosage forms.

Materials and Methods: A reverse phase (RP)-HPLC method for the simultaneous analysis of paracetamol, ACA, and ascorbic acid and their degradation and impurity products such as salicylic acid (SA) and p-chloroacetanilide was developed and applied to the determination of these compounds in commercial dosage forms. These compounds were well separated on a Bondapak C₁₈ reverse phase column using a mobile phase consisting of a mixture of methanol: water (35:65; v/v) adjusted to pH 3.1 with 10% orthophosphoric acid at a flow rate of 1.8 ml.min⁻¹ and the effluent was monitored at 235 nm. Sulfamethoxazole was used as an internal standard.

Results: The proposed method was linear in the ranges of 0.5-4.0, 0.75-6.0, 0.75-6.0, 1.0-12.0 and 1.0-12.0 µg ml⁻¹ for paracetamol, ACA, ascorbic acid, SA and p-chloroacetanilide, respectively. Relative standard deviations for repeatability, reproducibility and recovery were below 2%.

Conclusions: In this study, a RP-HPLC method, which is simple, rapid and does not require any separation step for each drug, was successfully applied for the quantitative assay of paracetamol, ACA, ascorbic acid, and their degradation and toxic impurity products in commercial tablet dosage forms.

Key Words: Degradation and impurity products, acetylsalicylic acid, paracetamol, salicylic acid, p-chloroacetanilide, HPLC

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Bazı Farmasötik Dozaj Şekillerinde Bulunan Asetil Salisilik Asit, Parasetamol ve Bunların Yıkılma/Katışıklık Ürünlerinin Hızlı ve Eşzamanlı Bir HPLC Yöntemi ile Saptanması

Amaç: Ticari farmasötik şekiller içinde bulunan ilaç moleküllerinin ve saflıklarının saptanması, toksikolojik ve farmakolojik bakımdan önemlidir. Farmasötik şekillerin hazırlanması sırasında saflığı ve ilgili bozulma ürünlerinin izlenebilmesi için ileri metotlara ihtiyaç vardır. Bu çalışmanın amacı farmasötik dozaj şekillerinde parasetamol, asetil salisilik asit (ASA) ve bunların yıkılma/katışıklık ürünlerinin HPLC yöntemi ile hızlı ve eşzamanlı olarak saptanmasıdır.

Yöntemler: Ticari dozaj şekillerindeki parasetamol, ASA, askorbik asit ve bu preparatlarda yıkılma ya da katışıklık ürünü olarak bulunan salisilik asit ve p-kloroasetanilidin saptanması için bir reverse faz HPLC (yüksek performanslı sıvı kromatografisi) metodu geliştirilmiştir. Bu bileşikler C₁₈ ters faz kolon kullanılarak mobil fazı metanol:su (35:65;v/v) karışımından oluşan pH'sı % 10'luk ortofosforik asitle 3.1'e ayarlanan akış hızı 1.8 ml/dakika ve 235 nm'de izlenmiştir. Sülfametaksazol internal standart olarak kullanılmıştır.

Bulgular: Yeni geliştirilen HPLC metodu, parasetamol, asetilsalisilik asit, askorbik asit, salisilik asit ve p-kloroasetanilid için sırasıyla 0.5-4.0, 0.75-6.0, 0.75-6.0, 1.0-12.0 ve 1.0-12.0 mg/ml aralıklarında doğrusal bulunmuştur. Tekrarlanabilirlik ve geri kazanım için göreceli standart sapma % 2'nin altında olarak saptanmıştır.

Sonuç: Bu çalışmada geliştirilen RP-HPLC metodu, parasetamol, ASA, askorbik asit içeren farmasötik dozaj şekillerinde ilaç molekülleri ve bunların bozulma ve/veya katışıklık (safsızlık) ürünleri için hızlı, basit ve herhangi bir ön ekstraksiyon gerektirmeyen ve başarıyla uygulanabilecek bir yöntemdir.

Anahtar Sözcükler: Yıkılma ve Katışıklık Ürünleri, Asetilsalisilik Asit, Parasetamol, Salisilik Asit, p-kloroasetanilid, HPLC

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Introduction

An impurity in a drug is any chemical component of the drug substance that has no chemical entity defined as a drug substance. The safety of a drug is dependent not only on the toxicological properties of the active substance itself, but also on its pharmaceutical impurities, which consist of reaction by-products generated during synthesis of drug substances (namely, active pharmaceutical ingredients) and degradation products formed during the formulation manufacturing process and/or storage of drug substances or formulated products. Pharmaceutical impurities, also referred to as 'related substances', could often have pharmacological or toxicological relevance. Therefore, the presence of such impurities and their levels in products are indicators of product quality, which can impose a risk to patient safety (1-3).

Acetylsalicylic acid (ACA) has analgesic, anti-inflammatory and antipyretic properties. It is used for relief of less severe types of pain such as headache, dysmenorrhea, myalgia and toothache (4,5). However, intentional or accidental ingestion of salicylates represents a major poisoning problem (6). Most salicylate poisoning involves the use of aspirin or ACA. Paracetamol (PAR) is also an analgesic and antipyretic drug but lacks anti-inflammatory properties (4,5). PAR is hepatotoxic and nephrotoxic in humans and experimental animals in toxic doses. PAR is one of the most common causes of morbidity and mortality in drug-poisoning cases (7-10).

Ascorbic acid (AA) is a water-soluble vitamin. It is essential for the synthesis of collagen and intracellular material (4,5). PAR, ACA and AA are active compounds that are widely used and frequently combined in pharmaceuticals. A combination of PAR, ACA and AA has been used in many formulations in the market.

Various analytical techniques for the determination of PAR, ACA and AA exist individually or in combination including liquid chromatography (11-18), spectrophotometry (11,18-22), spectrofluorometry (23), FT-Raman spectroscopy (24), and near infrared spectroscopy (25). High performance liquid chromatography (HPLC) methods are useful for simultaneous determination of drugs and their degradation and impurity products in pharmaceutical dosage forms. Since HPLC has been used widely in routine analysis, it is important to develop a HPLC method that is thoroughly validated (26-28).

To our knowledge, there is no information in the literature and pharmacopoeias about the simultaneous determination of PAR, ACA, AA and their degradation and impurity products, namely salicylic acid (SA) and p-chloroacetanilide (PCA), in bulk form and pharmaceutical formulations.

An ideal stability-indicating HPLC method should be able to resolve the main compounds of drug degradation and impurity products. Therefore, we have developed a rapid, accurate, reproducible and validated method for simultaneous determination of PAR, ACA and AA for their impurity and degradation products, namely SA and PCA.

Materials and Methods

Apparatus

A liquid chromatographic system consisted of a Waters Isocratic LC pump 510, with an automatic sample injection system (Waters 717 plus Autosampler), equipped with a Waters 996 photodiode array detector. Chromatographic separation was performed on a Bondapak C₁₈ reverse phase column packed with 10 mm dimethyloctadecyl bonded amorphous silica (300 mm × 3.9 mm). All solutions were filtered through 0.45 mm Millipore filter prior to use and degassed using an ultrasonic bath.

Chromatographic Conditions

The mobile phase consisted of a mixture of methanol: water (35:65; v/v) adjusted to pH 3.1 with 10% orthophosphoric acid. The mobile phase was prepared daily, filtered, and sonicated prior to use. All analyses were performed under isocratic conditions at a flow rate of 1.8 ml min⁻¹ and the effluent was monitored at 235 nm. 10 µl of each solution was injected and chromatograms were recorded.

Chemicals and Reagents

PAR, ACA, AA, SA and PCA were supplied from Sigma. Methanol was of HPLC grade, purchased from Merck (Darmstadt, Germany). All other chemicals were commercial analytical reagent grade. Double distilled water was used for preparing mobile phase solutions.

Standard Stock Solution

Stock solutions were prepared separately by dissolving PAR, ACA, AA, SA and PCA in mobile phase to obtain concentrations of 1.0, 1.5, 1.5, and 1.0 mg ml⁻¹,

respectively. The standard solutions of PAR, ACA, AA, SA and PCA containing a fixed concentration ($8 \mu\text{g ml}^{-1}$) of sulfamethoxazole (internal standard) (SMZ) were prepared in mobile phase by varying the different concentrations in the ranges of 0.5-4.0, 0.75-6.0, 0.75-6.0, 1.0-12.0 and 1.0-12.0 $\mu\text{g ml}^{-1}$, respectively. Triplicate 10 μl injections were made for each concentration and the peak area ratio of each drug to the internal standard was plotted against the corresponding concentration to obtain calibration graph.

The proposed method was validated, and precision [reported as the relative standard deviation (RSD %)], linearity (evaluated by regression equations), detection and quantification limits and accuracy were calculated. The limit of detection (LOD) and limit of quantification (LOQ) of the procedure are also shown in Table 1, which were calculated according to the 3 s/m and 10 s/m criterion, respectively, where s is the standard deviation of the peak area ratios (n=4) of the sample and m is the slope of the corresponding calibration curve.

The ruggedness and precision were checked on the same and different days. The RSD % was calculated to check the ruggedness and precision of the methods. Accuracy was determined by recovery studies.

Procedure for Tablets

Ten tablets (Afebryl effervescent tablet, SMB Technology, Belgium), labeled as containing 200 mg of PAR, 300 mg of ACA and 300 mg AA, and excipients were weighed and finely powdered. The average weight per tablet was calculated from the weight of 10 tablets. An accurate weight of the powder equivalent to one tablet content was accurately weighed and transferred into 100 ml calibrated flask, diluted with mobile phase, stirred and then made up to volume with the same solvent and filtered. Appropriate volume of the filtered solution was

taken in a 10 ml clean flask. Appropriate amount of internal standard was added and diluted up to the mark with the mobile phase.

Recovery Studies

To determine the accuracy, precision, reproducibility and interference from excipients in the formulation, recovery experiment of the above method was performed from the laboratory made mixtures.

Drug analysis has been undertaken during various phases of pharmaceutical development stages such as formulation and stability studies, quality control and pharmacological testing in animals and humans. All these investigations require reliable and fully validated analytical methods in order to determine active ingredients in pharmaceutical formulations and biological samples.

In order to determine the effect of the simultaneous elution of PAR, ACA, AA, SA and PCA peaks under isocratic conditions, the mixtures of methanol and water in different combinations, at different pH values and at various flow rates were examined. The mixture of methanol:water (35:65; v/v) adjusted to pH 3.1 with 10% orthophosphoric acid at 1.8 ml min^{-1} flow rate proved to be better than the other mixtures and flow rates for the separation since the chromatographic peaks were observed to be better and without tailing.

Results

The precision and accuracy can often be enhanced by the use of an appropriate internal standard, which also serves to correct for fluctuations in the detector response. Often, sample preparation steps that include reaction, filtration, precipitation, extraction and so on result in sample losses. When added prior to sample preparation, a proper internal standard should be used to

Table 1. Results of system suitability.

Parameters	PAR	ACA	AA	SA	PCA
Resolution factor	5.86	5.47	3.88	3.36	10.53
Tailing factor	1.67	1.17	1.14	1.50	1.28
Selectivity	1.99	1.62	1.74	1.26	1.72
Capacity factor	2.33	7.26	1.34	9.15	12.36
RSD% of retention time	0.96	1.29	1.14	0.86	1.21

PAR: Paracetamol. ACA: Acetylsalicylic acid. AA: Ascorbic acid. SA: Salicylic acid. PCA: p-Chloroacetanilide. RSD: Relative standard deviation.

correct for these losses. The maximum absorption for acetaminophen was at 235 nm; therefore, this wavelength was used throughout the analysis. The internal standard is a different compound from the analyte, but one that is well resolved in the separation. The chemical structure of SMZ is not similar to the other compound structures. However, it was chosen as the internal standard because it showed a shorter retention time with symmetrical peak and sharp and better resolution compared to other potential internal standards. SMZ did not interfere with the elution pattern of the other agents. The selected conditions yielded satisfactory chromatographic peak resolutions in a short analysis time. The retention times were determined as 1.87 min for AA, 2.66 min for PAR, 6.6 min for ACA, 8.12 min for SA, 13.36 min for PCA and 4.39 min for internal standard (SMZ) (Figure).

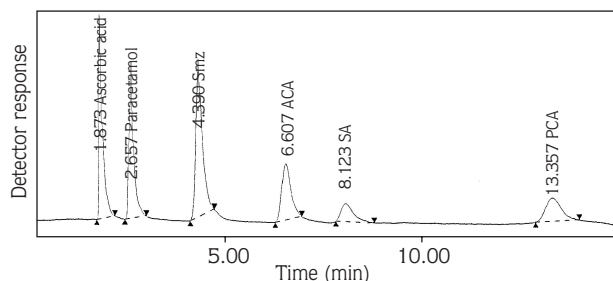


Figure. A typical chromatogram of an analysis of paracetamol (PAR), acetylsalicylic acid (ACA), ascorbic acid (AA) and their degradation and impurity products salicylic acid (SA) and p-chloroacetanilide (PCA), and internal standard sulfamethoxazole (SMZ).

Prior to the analysis of samples each day, the operator must establish whether HPLC system and procedure are capable of providing data of acceptable quality. This is accomplished with system suitability experiments, which can be defined as tests to ensure that the method can generate results with acceptable accuracy and precision. System suitability tests are used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis. System suitability tests were carried out according to United States Pharmacopoeia (USP) 24, method <621> (29) on the chromatogram of freshly prepared standard solutions to check various parameters. These parameters included relative standard deviations (RSD %) of retention times, tailing factor, resolution, capacity factor, selectivity factor and RSD % of peak height or area for repetitive injections and are shown in Table 1. Typically, at least two of these

criteria are required to demonstrate system suitability tests were carried out using freshly prepared standard stock solutions. As shown in Table 1, the method has enabled good resolution factors of adjacent peaks, which were greater than 1.0. The retention times in mobile phase were used as hold-up time for the calculation of capacity factor. The presented chromatographic conditions ensure adequate retentions for all compounds, since successful capacity factor values were obtained. The results obtained from system suitability tests are in agreement with the USP requirements.

Several approaches are given in the ICH guideline to determine the detection (LOD) and quantification (LOQ) limits (visual evaluation, signal-to-noise and standard deviation of the response and the slope of the corresponding calibration curve (LOD=3s/m; LOQ=10 s/m).

Peak area ratios ($A_{\text{sample}} / A_{\text{IS}}$) were plotted against corresponding concentrations in the different concentration ranges of the compounds. Linear regression parameters of the peak area ratios versus concentrations of the compounds are presented in Table 2. The results showed highly reproducible calibration curves with correlation coefficients of >0.999. Necessary statistical data of the regression equations such as LOD, LOQ values, repeatability and reproducibility data are also shown in Table 2. Repeatability and reproducibility were characterized by RSD % (Table 2). According to these results, there was no significant difference for the assay, which was tested within day (repeatability) and between days (reproducibility) (Table 2) (30,31). The proposed method also demonstrates good precision, accuracy and reproducibility, as shown in Table 2.

In order to demonstrate the validity and applicability of the proposed HPLC method, recovery tests were carried out by analyzing the synthetic mixtures of these compounds, which were reproduced in different composition ratios (Table 3). Recovery experiments using the developed assay procedure further indicated the absence of interference from commonly encountered pharmaceutical excipients used in the selected formulation and degradation product and impurity (Table 3).

The utility of the proposed method was verified by means of replicate estimations of the marketed product and results obtained were evaluated statistically (Table 4). It is concluded that the proposed method is

Table 2. Results of least square regression analyses for the estimation of PAR, ACA, AA, SA and PCA using the proposed method.

Statistical Parameters	PAR	ACA	AA	SA	PCA
Linearity range ($\mu\text{g MI}^{-1}$)	0.5-4.0	0.75-6.0	0.75-6.0	1.0-12.0	1.0-12.0
Correlation coefficient	0.999	0.999	0.999	0.997	0.999
Slope	0.512	0.249	0.106	0.128	0.470
Intercept	0.010	-0.018	-0.02	0.041	0.033
SE of slope	0.0046	0.0026	0.0022	0.0060	0.011
SE of intercept	0.001	0.0098	0.0081	0.004	0.0076
LOD ($\mu\text{g mL}^{-1}$)	0.036	0.048	0.130	0.107	0.038
LOQ ($\mu\text{g mL}^{-1}$)	0.119	0.161	0.432	0.358	0.128
Repeatability (RSD %)	0.45	1.25	1.03	0.695	0.76
Reproducibility (RSD %)	0.56	1.61	1.44	1.45	0.86

PAR: Paracetamol. ACA: Acetylsalicylic acid. AA: Ascorbic acid. SA: Salicylic acid. PCA: p-Chloroacetanilide. LOD: Limit of detection. LOQ: Limit of quantification. RSD: Relative standard deviation.

Table 3. Simultaneous determination of PAR, ACA and AA in the presence of their degradation products (SA and PCA) in laboratory-prepared mixtures using the proposed method.

Compound	Concentration ($\mu\text{g MI}^{-1}$)	Amount Found* ($\mu\text{g MI}^{-1}$)	Recovery* %	RSD % of Recovery
PAR	20.0	20.00	100.0	0.72
ACA	30.0	29.98	99.93	0.40
AA	30.0	29.96	99.87	0.60
SA	2.0	1.987	99.35	0.10
PCA	2.0	1.996	99.80	0.25

PAR: Paracetamol. ACA: Acetylsalicylic acid. AA: Ascorbic acid. SA: Salicylic acid. PCA: p-Chloroacetanilide. RSD: Relative standard deviation.

* Each value is the mean of five experiments.

Table 4. Determination of PAR, ACA and AA in the presence of their degradation products (SA and PCA) in pharmaceutical dosage form using the proposed method.

Compound	Labeled Claim (mg per tablet)	Amount Found %	RSD % of Amount Found
PAR	200.0	100.60	0.20
ACA	300.0	99.80	0.26
AA	300.0	99.96	0.16
SA	-	Not Detected	-
PCA	-	Not Detected	-

PAR: Paracetamol. ACA: Acetylsalicylic acid. AA: Ascorbic acid. SA: Salicylic acid. PCA: p-Chloroacetanilide. RSD: Relative standard deviation.

sufficiently accurate and precise for application to analysis of pharmaceutical dosage forms.

Conclusion

In the present study, we have shown that our proposed method using HPLC is convenient for

simultaneous detection of PAR, ASA, AA, SA and PCA. The results of this reliable HPLC method demonstrate a high level of precision and enough sensitivity for the determination of all five compounds simultaneously. The present HPLC method is simple, accurate and precise and can be used for the determination of PAR, ACA, and AA and possible degradation and impurity products in tablet

dosage forms. Thus, this procedure can be easily adopted for routine quality control analysis of tablet dosage forms without any interference from the excipients or each other.

Previously, several studies reported simultaneous detection of PAR and ASA (32) and plus SA (33). PAR and its impurity product PCA were also analyzed simultaneously using HPLC (34). However, PAR and ASA

and their impurity products plus AA have not been analyzed simultaneously until now. In some of the analgesics, ASA and PAR exist together with AA. Therefore, our proposed method for simultaneous detection of these five substances is important for quality control laboratories where economy and time are essential.

References

1. U.S. Food and Drug Administration; Guidance for Industry, Q3A Impurities in New Drug Substances 2003.
2. U.S. Food and Drug Administration; Guidance for Industry, Q3B Impurities in New Drug Products 2006.
3. Liu DQ, Wu L, Sun M, MacGregor PA. On-line H/D exchange LC-MS strategy for structural elucidation of pharmaceutical impurities. *J Pharm Biomed Anal* 2007; 44: 320-9.
4. Sweetman SC. Martindale, The Complete Drug Reference. 33rd ed. London: Pharmaceutical Press; 2002.
5. Hardman JG, Limbird LE. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 9th ed. New York: Mc Graw-Hill Companies; 1996.
6. Klaassen CD. Casarett & Doull's Toxicology: The Basic Science of Poisons. 5th ed. New York: McGraw-Hill Companies; 1996.
7. Al-Mustafa ZH, Al-Ali AK, Qaw FS, Abdul-Cader Z. Cimetidine enhances the hepatoprotective action of N-acetylcysteine in mice treated with toxic doses of paracetamol. *Toxicology* 1997; 121: 223-8.
8. Jaeschke H, Knight TR, Bajt ML. The role of oxidant stress and reactive nitrogen species in acetaminophen hepatotoxicity. *Toxicol Lett* 2003; 144: 279-88.
9. Stern ST, Bruno MK, Hennig GE, Horton RA, Roberts JC, Cohen SD. Contribution of acetaminophen-cysteine to acetaminophen nephrotoxicity in CD-1 mice: I. Enhancement of acetaminophen nephrotoxicity by acetaminophen-cysteine. *Toxicol Appl Pharmacol* 2005; 202: 151-9.
10. Thomas SH. Paracetamol (acetaminophen) poisoning. *Pharmacol Ther* 1993; 60: 91-120.
11. Ustun M, Sungur S, Ersoy L. Comparison of HPLC and derivative spectrophotometric method of the determination of paracetamol (acetaminophen) and acetylsalicylic acid in tablets. *Pharmazie* 1992; 47: 558-9.
12. Tebbett IR, Omile CT, Danesh B. Determination of paracetamol, salicylic acid and acetyl salicylic acid in serum by high-performance liquid chromatography. *J Chromatogr* 1985; 329: 196-8.
13. Baum RG, Cantewell FF. Determination of salicylic acid and aspirin in multicomponent tablets by liquid chromatography on a nonionic resin. *J Pharm Sci* 1978; 67: 1066-9.
14. el Sadek M, el Shanawany A, Aboul Khier A, Rucker G. Determination of the components of analgesic mixtures using high-performance thin-layer chromatography. *Analyst* 1990; 115: 1181-4.
15. Pufal E, Sykutera M, Rochholz G, Schutz HW, Sliwka K, Kaatsch HJ. Determination of paracetamol (acetaminophen) in different body fluids and organ samples after solid-phase extraction using HPLC and an immunological method. *Fresenius J Anal Chem* 2000; 367: 596-9.
16. Ramos-Martos N, Aguirre-Gomez F, Molinz-Diaz A, Capitan-Vallvey LF. Application of liquid chromatography to the simultaneous determination of acetylsalicylic acid, caffeine, codeine, paracetamol, pyridoxine and thiamine in pharmaceutical preparations. *J AOAC Int* 2001; 84: 676-83.
17. Abu-Qare AW, Abou-Donia MB. A validated HPLC method for the determination of pyridostigmine bromide, acetaminophen, acetylsalicylic acid and caffeine in rat plasma and urine. *J Pharm Biomed Anal* 2001; 26: 939-47.
18. Vidal AD, Barrales PO, Diaz AM. Simultaneous determination of paracetamol, caffeine and propyphenazone in pharmaceuticals by means of a single flow-through UV multiparameter sensor. *Microchim Acta* 2003; 141: 157-63.
19. Toral MI, Richter P, Araga E. Determination of mefenamic acid and paracetamol by first derivative spectrophotometry. *Anal Lett* 1996; 29: 2679-89.
20. Vidal AD, Reyes JFG, Barrales PO, Diaz AM. UV spectrophotometric flowthrough multiparameter sensor for the simultaneous determination of acetaminophen, acetylsalicylic acid and caffeine. *Anal Lett* 2002; 35: 2433-47.
21. Criado A, Cardenas S, Gallego M, Valcarcel M. Continuous flow spectrophotometric determination of paracetamol in pharmaceuticals following continuous microwave assisted alkaline hydrolysis. *Talanta* 2000; 53: 417-23.
22. Nogowska M, Muszalska I, Zajac M. Simultaneous spectrophotometric determination of acetylsalicylic acid, paracetamol and caffeine in pharmaceutical preparations. *Chem Anal Warsaw* 1999; 44: 1041-8.
23. Martos NR, Diaz AM, Navalon A, Capitan-Vallvey LF. Spectrofluorimetric determination of acetylsalicylic acid and codeine mixtures in pharmaceuticals. *Anal Lett* 2001; 34: 579-95.

24. Szostak R, Mazurek S. Quantitative determination of acetylsalicylic acid and acetaminophen in tablets by FT-Raman spectroscopy. *Analyst* 2002; 127: 144-8.
25. Merckle P, Kovar KA. Assay of effervescent tablets by near-infrared spectroscopy in transmittance and reflectance mode: acetylsalicylic acid in mono and combination formulations. *J Pharmaceut Biomed* 1998; 17: 365-74.
26. Uslu B, Özkan SA. Determination of lamivudine and zidovudine in binary mixture using first derivative spectrophotometric, first derivative of the ratio spectra and HPLC-UV methods. *Anal Chim Acta* 2002; 466: 175-85.
27. Özkan SA, Uslu B. A rapid HPLC assay for the determination of lamivudine in pharmaceuticals and human serum. *J Liq Chrom Rel Tech* 2002; 25: 1447-56.
28. Pham-Huy C, Stathouloupoulou F, Sandouk P, Scherrmann JM, Palombo S, Girre C. Rapid determination of valaciclovir and acyclovir in human biological fluids by high-performance liquid chromatography using isocratic elution. *J Chromatogr B Biomed Sci Appl* 1999; 732: 47-53.
29. Taunton MA. *The United States Pharmacopoeia*. 24th rev. Easton: Rand Mc Nally; 2000.
30. Riley CM, Rosanske TW. *Development and Validation of Analytical Methods*. New York: Elsevier Science Ltd.; 1996.
31. Swartz ME, Krull IS. *Analytical Method Development and Validation*. New York: Marcel Dekker; 1997.
32. Franeta JT, Agbaba D, Eric S, Pavkov S, Aleksic M, Vladimirov S. HPLC assay of acetylsalicylic acid, paracetamol, caffeine and phenobarbital in tablets. *Farmaco* 2002; 57: 709-13.
33. Ramos-Martos N, Aguirre-Gómez F, Molina-Díaz A, Capitán-Vallvey LF. Application of liquid chromatography to the simultaneous determination of acetylsalicylic acid, caffeine, codeine, paracetamol, pyridoxine, and thiamine in pharmaceutical preparations. *J AOAC Int* 2001; 84: 676-83.
34. Elbarbry FA, Mabrouk MM, El-Dawy MA. Determination of the analgesic components of Spasmomigraine tablet by liquid chromatography with ultraviolet detection. *J AOAC Int*. 2007; 90: 94-101.