

Ion Chromatographic Determination of Colchicine, Ampicillin and Paracetamol in Pharmaceutical Preparations and Urine Matrix.

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Abstract

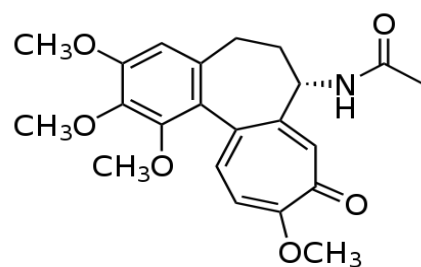
A new, simple, rapid and inexpensive ion chromatographic method with UV detection has been established for the separation and simultaneous determination of colchicine, ampicillin and paracetamol in pure form and pharmaceutical preparations individually and in mixture. The method is based on using of weak and strong cation exchanger columns (WCX and SCX). The retention mechanisms for the selected drugs for both columns have also been investigated; thereby their retention parameters were quantified at optimum conditions. The ion chromatographic method was validated for linearity, limit of detection, limit of quantification, precision and recovery percent. Linearity was found in the range of 5-200 mg L⁻¹ for colchicine and of 2.5-200 mg L⁻¹, for ampicillin and paracetamol, with detection limits of, 0.92, 2.08 and 2.06 mg L⁻¹ and recovery percent of 94±0.05%, 99.95±0.5% and 104.8±4.6%, for colchicine, ampicillin and paracetamol respectively. The proposed method was successfully applied for the direct determination of colchicine in urine sample using "dilute and shoot" approach.

Key Words

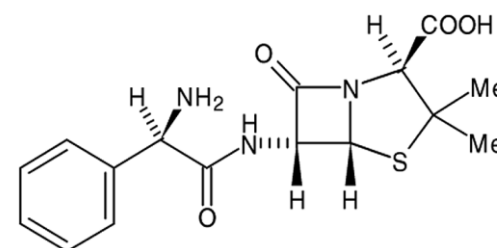
Colchicine; ampicillin; paracetamol; ion chromatography; dilute and shoot approach; urine sample.

Introduction

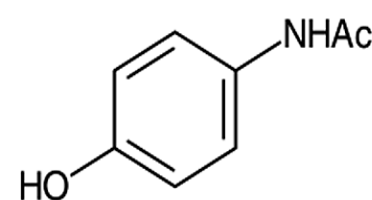
Colchicine (Figure .1a) is an alkaloid drug, chemically known as N-[(7*S*)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl] acetamide, and widely used for the treatment of gout disease Ampicillin (Figure.1b) is an antibiotic, chemically known as [2*S*-[2α, 5α, 6β(*S**)]]-6-[(Aminophenylacetyl) amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid, used to treat many different types of infections caused by bacteria, such as ear infections, bladder infections, pneumonia, gonorrhea, and E. coli or salmonella infection. Paracetamol or acetaminophen (Figure.1c) is an analgesic and antipyretic, chemically known as *N*-(4-hydroxyphenyl) acetamide and commonly used for the relief of fever, headaches, and other minor aches and pains¹.



(a)



(b)



(c)

Figure 1: Chemical structure of (a) colchicine, (b) ampicillin and (c) paracetamol.

The misuse of two or more drugs is frequent or sometime happens that patients are treated simultaneously with a few drugs representing different groups. These, in fact leading to several undesirable consequences due to interaction between drugs that one drug may increase or decrease the effect of another². In this respect, an analgesic and antipyretic drugs such as, paracetamol or aspirin decrease colchicine effectiveness, while such antibiotics in combination with gout drug colchicine

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at the same time increase the risk of their side effects. For example, Beta-lactam antibiotics cause psychic disturbances to the patient and fatal cases are also well known due to the use of clarithromycin, erythromycin in combination with colchicine treatment which leads to colchicine toxicity³. Causes of abuse combination of drugs when have submitted to laboratories cause problems to the analysts in obtaining rapid and reliable results⁴. Consequently, standard and selective analytical protocol is a must, for the simultaneous separation and quantification of the drugs mixture in the biological samples for the purposes of quality control and more significantly for the clinical diagnosis. Literature survey reveals that many analytical methods are reported for determination of colchicine⁵⁻¹⁰, ampicillin¹¹⁻¹⁵, and paracetamol¹⁶⁻²⁷, individually or in combination with other related drugs. The techniques used for measurement of the selected drugs such as HPLC-MS, LC-ESI-MS and GC-MS, all require highly sophisticated instruments which are relatively expensive and hence are not always available. On the other hand, UV-Vis spectrophotometric methods are simple and offer appropriate precision and sensitivity, but suffer from strongly interferences of drug excipients and inaccurate for simultaneous determination of drug mixtures. Availability of ion chromatograph instrument in many laboratories and the simplicity of analytical procedures make the technique very attractive for wide range of applications, including the determination of inorganic ions in different matrices and analyses of organic compounds. A recent literature survey reveals that there are very scanty reports of using ion chromatographic method for simultaneous determination of medicaments in mixtures. This has encouraged the authors to develop simple, rapid, reliable and inexpensive method for the colchicine and ampicillin and paracetamol in their mixture and biological samples, to exploit IC technique in pharmaceuticals analyses and given opportunity to analyst to use this technique for the assay of drug mixture and in case of any state of emergency may occur to patients of misusing the drugs. This paper describes a simple, rapid, inexpensive and sensitive validated ion chromatographic method with UV detection for the separation and simultaneous determination of colchicine, ampicillin and paracetamol in individual pharmaceutical preparations, their mixtures and urine matrices. The

method is based on employing two cation exchanger columns ((WCX and SCX) to evaluate the best ion exchange mechanism behaviors of the selected drugs on the basis of the differences in the ionization of the analytes. The cation exchangers are either based on hydrophobic polystyrene with sulfonic acid groups (SCX-column) or on silica as a more hydrophilic backbone, which has been coated with a polybutadiene-maleic acid copolymer. The results also show that the proposed method is a useful tool for the diagnosis and monitoring colchicine in urine samples using "dilute and shoot" approach without using any clean-up procedure.

Experimental

Instrumentation

The chromatographic system is consisted of a Merck Hitachi HPLC pump model L-6200A, a Merck Hitachi UV-Vis detector model (L-4250), Merck Hitachi column thermostat (T-630), Gynkotec Degasser(DG-1310), Rheodyn injection valve model 7125 containing a 20- μ L and 50- μ L sample loop, and Merck Hitachi integrator model 2500. The system is equipped individually with two column; the homemade strong cation exchanger column type KSO2_213 (150 x 4 mm, 5 μ m) with equivalent capacity for H⁺ of 0.21 mmol/column which prepared and described elsewhere²⁸, and a commercial weak cation exchanger column type Metrohm C2 (150x4 mm, 7 μ m). The whole system is controlled by using a computer working with software type (IC Net 2.3, Metrohm). The optimized chromatographic conditions of both columns for separation of the three drugs are outlined in Table 1.

Chemicals and Reagents

Colchicine pure sample was obtained from Applichem (Germany), pure ampicillin was gifted from Biochemistry Department (Marburg University) and paracetamol (99.6-100%) from BDH. Analytical reagent- grade HCl (37%) from BDH and HPLC-grade acetonitrile (ACN) obtained from Acros Organics (New Jersey, USA). Millipore water was used throughout this study. The stock solutions of each pure drug (1 mg mL⁻¹) was prepared directly by dissolving of 100 mg in a mixture of mobile phase (0.1 M HCl: ACN, 1:1) and transferred to 100 mL volumetric flask and diluted to mark with same mobile phase. Stock solutions were stored in refrigerator and stable for at least one month.

Daily working solutions of each drug and the synthetic mixtures were prepared by suitable dilution of stock solution with mobile phase. Ten of the standard calibration mixtures were prepared in mobile phase in the range of (5-500 mg L⁻¹) for colchicine , (2.5-500 mg L⁻¹) for ampicillin and paracetamol and 20 µL was injected three times at optimum conditions into the column, the peak areas and retention times were recorded .

UV-Spectra of the Drugs

UV-spectra for the individual drug were recorded using Spektrophotometer, DMR 10(ZEISS) controlled with microcomputer for recoding the spectra at 10 mg L⁻¹ of standard solution colchicine and paracetamol and 100 mg L⁻¹ ampicillin to find out the wavelength maxima that is suitable chromatographic separation.

Analytical Procedure

Preparation of Pharmaceutical preparations

The tablets sample solutions for ampicillin and paracetamol were prepared using a method described elsewhere with some modifications ²⁹ . The 5 tablets of ampicillin and 10 tablets consisting of paracetamol were crushed individually in a porcelain mortar. An amount equivalent to the stated value was weighted into 50 mL beaker and 25 mL of 0.1M HCl was added. The mixture is shaken at magnetic stirrer for two hours. The solution was filtered and passed through Whatmann 598/1 filter. The filtrate was transferred to 100 mL volumetric flask and adjusted the volume to mark with HCl (0.1 M). The prepared extract was further diluted as required with mobile phase to fall with the calibration concentrations range. An amount of 20 µL was injected into LC column and the amount of active substance was calculated from regression equation. For colchicine, 10 tablets were pulverized in a porcelain mortar and an amount equivalent to the stated value of the active substance was transferred into 10 mL beaker, then 2 mL of HCl (0.1 M) was added. The mixture is put in an ultrasonic bath at room temperature for an hour in order to solubilize the active substance. The mixture is then filtered into 10 mL volumetric flask and the filtered is adjusted to mark with mobile phase. An amount of 20 µL was injected into LC column and the amount of active substance was calculated from regression equation.

Preparation of urine sample

The drug-free urine sample was collected from normal volunteer and kept freeze in the refrigerator until analysis. The urine sample was thawed and an amount of 200 µL was transferred into a series of 10 mL volumetric flasks and spiked with varying concentrations of colchicine in the range of 10-40 mg L⁻¹, then 100 µL of HCl (0.1M) were added. The content of the flasks were shaken for five minutes and completed to mark with Millipore water. The contents were then transferred into a series of 10 mL of centrifugation tubes and centrifuged for 10 min at 8000 rpm. The blank urine was treated in the same manner. Aliquots of 20 µL were injected in triplicate into WCX column and calibration graph was constructed. In a separated experiment, samples of spiked urine containing the same amounts of colchicine were analyzed directly and % recoveries were calculated from the regression equation.

Results and Discussion

Preliminary study

The UV spectral characteristics of the three pharmaceuticals in their pure solutions were previously studied and they are shown in Figure.2. These spectra were obtained using cell of 10 mm optical path length. The maximum absorbance wavelengths were 245 nm for colchicine and paracetamol and 225 nm for ampicillin. We chose 230 nm for compromise as a wavelength maximum for separation and simultaneous determination of these analytes using the peak areas as analytical chromatographic signals.

Optimization of Chromatographic Conditions

Main reason for the selection of cation exchange chromatography as a tool for the separation of the selected drugs was the protonable nitrogen group in ampicillin, which offers selectivity tunable by ion exchange retention. We expected a mixed mode cation exchange and reversed phase retention for this analyte. The other analytes colchicine and paracetamol are very weak bases (pKa < 0) with a pure hydrophobic retention on cation exchangers. Figures 3 to 6 show the results of the experiments for the quantification of the ion exchange and hydrophobic retention behavior for each analyte on the selected two types of cation exchangers. The determination of the retention properties of the analytes are based on the retention model of Haddad³⁰ for ion exchange and those of

Schoemakers³¹ for reverse phase retentions. Both models predict a double logarithmic correlation between the retention factor and the eluent compound, e.g. the ionic part for ionic retention or the stronger solvent for hydrophobic retention. The silica based weak cation exchanger shows ion exchange based on retention only for ampicillin as can be seen from Figure 3. The effective charge of the molecule can be calculated to be +0.47 assuming an effective charge of +1 for hydronium cation. The pKa values of ampicillin are 7.3 for the amino and 2.5 for the carboxyl group. The pH of the experiments for the WCX column varied from 2.2 to 3, therefore a protonation degree of around 50% is consistent with the molecule properties.

The behavior of the SCX column shown in Figure 3 is quite similar to that of the WCX column (Figure 4) in terms of ionic retention. The higher HCl concentration and therefore the lower pH increase the ionization of ampicillin, which is evident from the increased slope in Figure 3. The comparison of the SCX and WCX columns as shown in Figures 5 and 6 revealed that the styrene polymer backbone the SCX column leads to much stronger hydrophobic retention.

Main reason for the selection of the WCX column as best suited one for the separation of the three drugs is the higher separation efficiency of the WCX column together with the overall lower eluent concentration of both organic modifier and ionic compound. The lower efficiency of hypercrosslinked polymer based cation exchangers compared to silica gel based ones is caused by the broad pore size distribution of macroporous polymers. We have observed an inversion of the retention order due to increased ion exchange based on retention on column II (WCX column). The only anionic analyte ampicillin is more strongly retained on the strong acidic sulfonic acid group. The retention data for the selected drugs are summarized in Table 2.

Analytical Characteristics

Calibration curves were obtained for simultaneous determination of the three analytes by injection of 20 μL of standard analytes mixture into WCX column at optimized conditions (Table 1). The analytical figures of merit which statistically obtained were summarized in Table 3. Very good linearity was obtained in concentration range 5-200 mg L^{-1} for colchicine and 2.5-200 mg L^{-1} for ampicillin and paracetamol with coefficient of determination (R^2) of

more than 99.96% which suggests a statistically valid fit³². We use this fitted linear calibration model to estimate the analytes concentration in the drug samples which appears justified, on statistical basis.

The detection and quantification limits were estimated directly from regression using DIN program and was found to be (0.92 and 2.08 and 2.06 mg L^{-1}) and (1.84, 5.67 and 4.11 mg L^{-1}) for colchicine and ampicillin and paracetamol respectively. While the detection and quantification limits that determined experimentally as the concentration of analyte that produced an analytical signal equal to three and ten times the standard deviation of the background signal were found to be (0.927, 0.150 and 0.123 mg L^{-1}) and (3.11, 0.49 and 1.23 mg L^{-1}) for colchicine, ampicillin and paracetamol respectively. The repeatability (RSD %) of the proposed method at the low and high concentration within the calibration range was (0.63-1.48%) for colchicine, (0.88-3.37%) for ampicillin and (0.83-1.98%) for paracetamol.

Determination of the drugs in Pharmaceutical preparations

The developed method was applied to the detection of the three analytes in one of their selected tablets, colchicine (Dispert[®] GmbH), ampicillin (ratiopharm[®] GmbH) and paracetamol (SRADA[®] GmbH) with stated values of 0.5, 1000 and 500 mg /unit respectively, by using direct calibration procedure. Table 4 shows the results of determination which revealed the results found were agreed with stated concentration value with the respect to colchicine, ampicillin and paracetamol with relative error percent of, (-6.00%) and (-0.05%) and (4.80%) respectively.

Recovery Test

Since the certificate reference materials (CRM's) for the determination of the analytes in drug samples is not available, accuracy has been tested through the recovery percent evaluation. Recoveries were in the range of 93.95- 94.05%, 99.45-100.45% and 100.2-109.4% for colchicine, ampicillin and paracetamol, respectively. The low recovery of colchicine may be affected by the presence of the other constituents in the tablet sample. The t-test statistics reveals that for recovery results in this study for ampicillin and paracetamol are in a good agreement with the work of Barot et al³³ and Avidya et al³⁴ who used HPLC technique for ampicillin and paracetamol respectively.

Determination of colchicine in urine

In an attempt to detect the three drugs simultaneously in urine sample, many procedures based on "dilute and shoot" approaches have been tried for direct inject of urine. It was shown that this approach is only acceptable for colchicine but not for ampicillin and paracetamol as the urine constituent peaks strongly overlap with these two analytes peaks at 1.92 and 3.93 min respectively. Consequently, a totally clean-up method of urine matrix is a must in this case and we believe that the solid-phase extraction (SPE) procedure is a good for this purpose. The proposed method was successfully applied for the in-vitro determination of colchicine in spiked urine with different concentrations within the working range of the calibration graph using "dilute and shoot" approach. The linearity in the spiked urine was good over the range 10-40 mg L⁻¹. The regression equation obtained from a standard series of spiked urine was,

$$P = 19.578 C + 14.066 \quad (r = 0.9977)$$

Where P is the peak area and C is the concentration of colchicine in mg L⁻¹ in the spiked urine. The detection and quantification limits for colchicine were estimated directly from regression using DIN program and was found to be 3.6 and 7.3 mg L⁻¹. The percentage recoveries from added amounts in urine are depicted in Table 5.

This excellent recovery may be ascribed to the good separation of colchicine peak with a retention time of 5.32 min compared with less than 2 min for those of urine matrix constituents as shown in Figure 7. The recovery results using IC and "dilute and shoot" approach was proved to be simple and rapid compared to the work of Qiu et al⁸ who used HPLC-ESI-MS with extraction recovery in the range of 96-106% for colchicine in urine.

Conclusions

This work presents for the first time the separation and simultaneous determination of three selected drugs in their mixture using ion chromatographic techniques. The proposed method is simple, rapid, reliable and inexpensive for the assay of colchicine, ampicillin and paracetamol individually in tablets formulations and/or in synthetic mixture. The cation exchange mechanism was chosen because of the differences in the ionization of the analytes. The pK_a values colchicine and paracetamol are below zero.

Only ampicillin (pK_{a1} = 2.5, pK_{a2} = 7.3) was expected to be cationic under the conditions used for cation separation in ion chromatography. The other analytes were hydrophilic and should be retained by a reversed phase mechanism on the cation exchanger substrate. The proposed method has shown to be useful for the determination of colchicine in urine sample without any considerable pretreatment by using "dilute and shoot" approach. However, further work is needed for a totally clean-up of urine matrix in case of the simultaneous determination of the three drugs in the biological samples by using, for example solid-phase extraction(SPE)procedure combined with ion chromatography.

Acknowledgment

This work supported by Scientific Qatar Grant, organized by UNESCO-DAAD Fellowship for short 3-months stay of Ass. Prof. Dr. Zuhair Khammas at Marburg University in Germany is gratefully acknowledged. Sincere appreciation is also extended to Professor Gernot Frenking of Fachbereich Chemie/ University of Marburg, for his hospitality in providing the work place with computer access in his work group.

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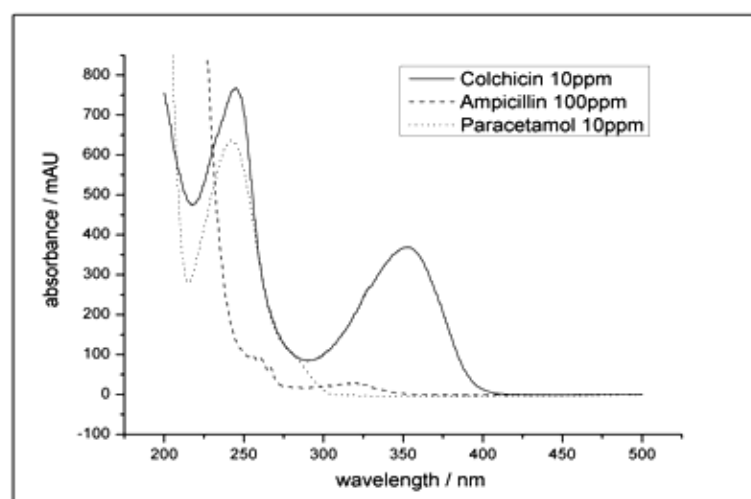


Figure 2: UV Spectra of colchicine (10 mg L^{-1}) ampicillin (100 mg L^{-1}) and paracetamol (10 mg L^{-1}).

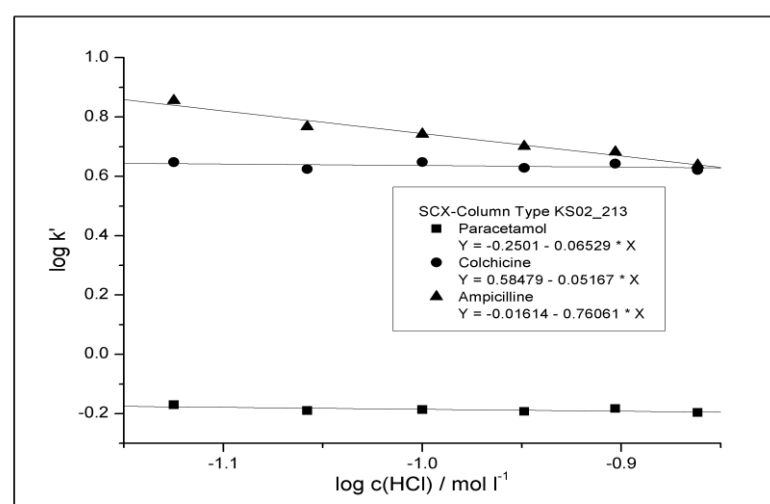


Figure 3: Variation of solute capacity factors with increasing concentration HCl using strong cation exchanger SCX homemade column.

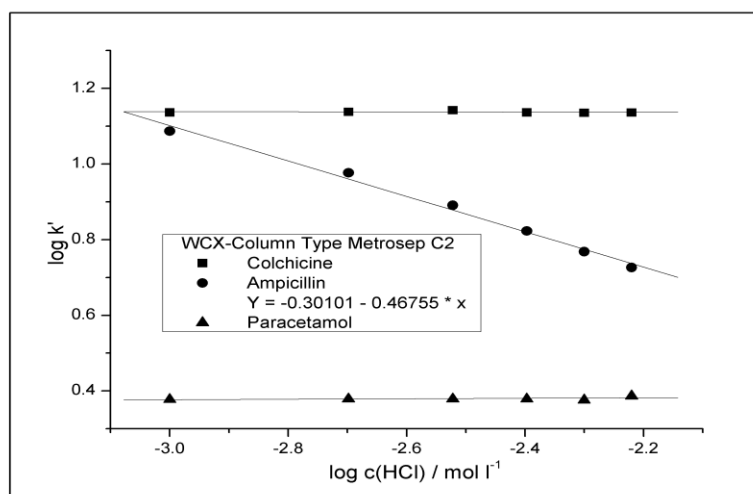


Figure 4: Variation of solute capacity factors with increasing concentration HCl using weak cation exchanger WCX column.

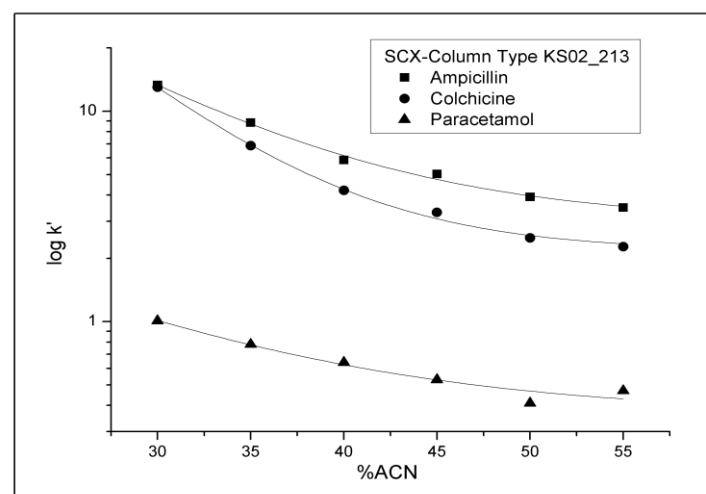


Figure 5: Variation of solute capacity factors with increasing % ACN using strong cation exchanger SCX homemade column.

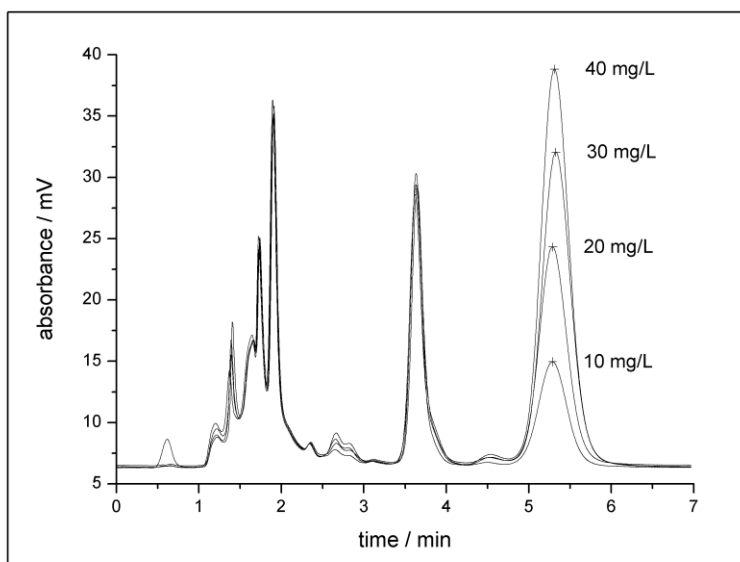


Figure 6: Typical chromatograms of dilute urine spiked with varying amounts of colchicine with concentrations of 10, 20, 30 and 40 mg L⁻¹ at t_R=5.32 min. Other peaks were not identified which belong to the urine matrix.

Table 1: Optimized chromatographic conditions for the separation and determination of colchicine, ampicillin and paracetamol in synthetic mixture.

Parameter	Column I	Column II*
Column type	WCX Metrohm C2(150 x 4 mm, 7 μm)	SCX KSO2_213 (150 x 4 mm, 5μm) with equivalent capacity of 0.21mmol/column.
Mobile Phase	15%ACN**,20% HCl (4 mmol) 65% H ₂ O	50%ACN, 35% HCl (0.25M) 15% H ₂ O
Flow rate	1.0 mL min ⁻¹	0.5 mL min ⁻¹
UV Detection	230 nm	230 nm
Injection volume	20 μL	50 μL
Column temperature	40 °C	40 °C

*Homemade, **Acetonitrile

Table 2: Retention data for colchicine, ampicillin and paracetamol for the column I and II at optimum conditions.

Compound	Column I					Column II				
	t_R min	k^{1a}	R_s^b	N^c	P_{assym}	t_R min	k^{1a}	R_s^b	N^c	P_{assym}
Colchicine	5.46	7.15	2.79	9863	1.22	6.29	2.86	5.91	6834	0.81
Ampicillin	3.98	4.94	7.77	16533	1.24	8.61	4.28	2.45	7831	0.58
Paracetamol	1.94	1.89	6.47	23780	1.55	2.55	0.56	1.57	4336	2.47

(a) capacity factor, (b) resolution, (c) theoretical plates.

Table 3: Representative statistical results for the analysis of the drug mixture by ion chromatography using column I.

Figures of merit	Colchicine	Ampicillin	Paracetamol
Concentration range (mg L ⁻¹)	5-200	2.5-200	2.5 – 200
Regression line (n=10)	y=2.33+21.24 x	y=3.07+1.24x	Y= 16.37+25.56 x
Correlation coefficient (r)	0.9998	0.9995	0.9998
Coefficient of determination (R ²)	99.96	99.90	99.96
C.L. for the slope(b±ts _b) at 95%	21.24±6.43	1.24±1.157	25.56±11.1
C.L. for the interception (a ± ts _a) at 95%	2.33±0.077	3.07±0.014	16.37±0.134
LOD (mg L ⁻¹)	0.92	2.08	2.06
LOQ (mg L ⁻¹)	1.837	5.665	4.112
*RSD% at 2.5 (mg L ⁻¹)	1.48	3.37	1.98
*RSD% at 200 (mg L ⁻¹)	0.63	0.88	0.83

Table 4: Analysis of tablet formulations by ion chromatography using column I.

Formulation	Labeled value(mg)	Amount found (mg)	%Recovery ±SD
Colchicine	0.5	0.47	94±0.05
Ampicillin	1000	999.5	99.95±0.5
Paracetamol	500	524	104.8±4.6

Table 5: % Recovery of colchicine from human urine using the proposed ion chromatographic method with column I.

Amount added mg L ⁻¹	amount found mg L ⁻¹	Recovery (%)	Mean ±C.L; p=0.05
10	9.43	94.30	98.20 ± 4.54
20	20.16	100.80	-
30	29.34	97.80	-
40	39.90	99.80	-
