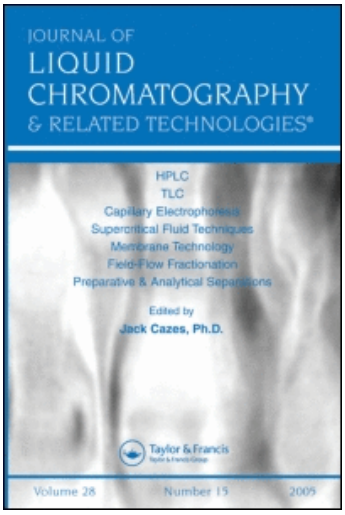

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Fast Screening of Chloramphenicol in Wastewater by High Performance Liquid Chromatography and Solid Phase Extraction Methods

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Abstract: Chloramphenicol is a classical antibiotic, used for the treatment of typhoid fever all over the world. Some potential manufacturers and users dealing with chloramphenicol are contaminating our natural water resources by discharging their effluents. Therefore, a sensitive, inexpensive, fast, and reproducible HPLC-SPE method was developed for the analysis of chloramphenicol in the wastewater. The column used was monolithic, chromolith performance RP-18e, 100–4.6 (100 × 4.6 mm). The mobile phase used was phosphate buffer (100 mM, pH 3.0)-acetonitrile (75:25, v/v) at 1.5 mL/min with UV detection at 275 nm. The retention, separation, and resolution factors of chloramphenicol were 3.13, 2.0, and 4.30, respectively. The percentage recovery of chloramphenicol from wastewater was 94.0%. Frusemide was used as the internal standard to access the percentage extraction of chloramphenicol from wastewater.

Keywords: Chloramphenicol, High performance liquid chromatography, Solid phase extraction, Wastewater

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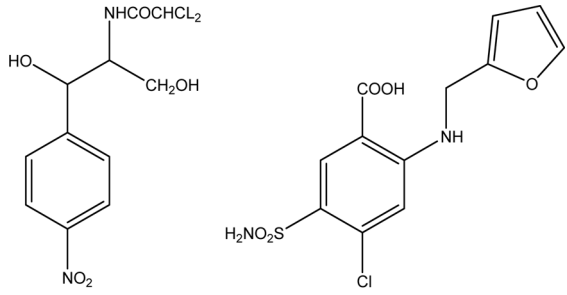
INTRODUCTION

Nowadays, many drug residues are present in waste, surface, and ground water and enter into the human body through water. The unnecessary administration of these drug residues into human body is not desirable and safe. The presence of drug residue in water resources may be considered as pollutants.

Analysis of drug residues in water is a recent area and increasing in its importance day by day.[1] The administration of these drug residues into the human body through water possess certain side effects and also alter the biological activities leading to notorious health effects.[2] Chloramphenicol is a classical antibiotic and used for the treatment of typhoid fever all over the world. Some potential manufacturers and users dealing with chloramphenicol are contaminating our natural water resources by discharging their effluents (wastewater or solid waste). Therefore, analysis of such a drug like chloramphenicol in wastewater is required to get accurate, quantitative information about its presence as a pollutant and is currently needed in order to protect society from its toxic effects. A search of literature indicates availability of many reports[3–10] of chloramphenicol analyses in different samples like blood, meat, milk, food, and urine, but the analysis of chloramphenicol in water or wastewater is still an undiscovered area. Therefore, development of an analytical method for the analysis of chloramphenicol in water samples is essential and required.

The conventional high performance liquid chromatographic methods involve the normal columns, and optimization of the analysis requires various complex procedures or numerous experiments leading to the consumption of large amount of costly chemicals, samples, and labor. Therefore, the speed of analysis is becoming important in many applications of high performance liquid chromatography. The running cost of high performance liquid chromatography can be reduced by decreasing the analysis time, which is urgent needed by the chromatographers. Recently, a special type of silica based column of high speed and economic analysis (the monolithic column), has been introduced into the market.[11,12] Some articles[13,14] and reviews[15,16] have also appeared claiming the fast and economic analysis by this column for a variety of molecules. However, to the best of our knowledge, no report is available on the analysis of chloramphenicol in wastewater by using this newly developed column. Therefore, attempts have been made to separate, identify, and quantify chloramphenicol antibiotic in wastewater by using the monolithic column in high performance liquid chromatography. For this study, frusemide was used as the internal standard. The structures of chloramphenicol and frusemide are given in Figure 1. The results of these analyses are described in the next section.



Chloramphenicol **Frusemide**

Figure 1. Chemical structures of chloramphenicol and frusemide.

EXPERIMENTAL

Materials and Methods

Chloramphenicol and frusemide were obtained from Sigma Chemical Co., USA. Purified water was prepared by Millipore Milli-Q (Bedford, M.A., USA). Acetonitrile, methanol, acetone, and acetic acid reagents were purchased from Merck, India. Sodium phosphate (Na_2HPO_4) was purchased from Fisher Scientific Co., USA. Sodium dihydrogen phosphate was supplied by B.D.H. Chemical Co., UK. Phosphate buffer (100 mM, pH 3.0) was prepared as per the standard procedure. The solutions (10.0 μg/mL) of the individual and the mixture of chloramphenicol and frusemide were prepared in methanol. pH was adjusted with a pH meter (Hach, Loveland Co.). SPE was carried out by using C_{-18} Sep-Pak Vac (1.0 mL) cartridge, which was obtained from Waters, USA. The HPLC system (Shimadzu, Japan) consisting of solvent delivery pump (model LC-10AD), injector (model SC), UV-Visible absorbance detector (model SPD-10A), and hp laser jet printer was used for this work. The HPLC monolithic silica column (100×4.6 mm) was obtained from Merck K_{ga} A, Darmstadt, Germany.

Methodology

Solid Phase Extraction

Solid phase extraction of chloramphenicol and frusemide was carried out as per the standard procedures. To determine the percentage recovery of chloramphenicol in the wastewater, frusemide was used as an internal standard. Solutions of chloramphenicol of 1.0 mL (1.0 mg/mL in

methanol) was mixed with 999.0 mL of tap water. This mixture was shacked for about 30 minutes and kept at room temperature over night. The $C\text{-}_{18}$ cartridge was preconditioned using methanol (1.0 mL) followed by water (1.0 mL). Of the spiked water sample, 1.0 L was passed through this cartridge at 50.0 mL/min flow rate. The cartridge was washed with 2.0 mL of deionized water and chloramphenicol was eluted with methanol (1.0 mL) thrice at 0.50 mL/min flow rate. Three fractions of eluted methanol were combined together and evaporated to 10 μL, which was injected on to an HPLC system. Besides, elution was also tried with other solvents such as acetone, ethanol, ethylacetate, hexane, and dichloromethane. This methodology was applied to the natural condition by replacing tap water with wastewater. The wastewater sample was collected from the municipal discharge and filtered through Whatman filter papers No. 24. The filtered wastewater sample (999.0 mL) was spiked with 1.0 mL chloramphenicol (1.0 mg/mL in methanol) and treated as in the case of tap water. Similarly wastewater samples were spiked with frusemide and an SPE extraction procedure was carried out to calculate the percentage recoveries of both chloramphenicol and frusemide.

Analysis by HPLC

Analysis of chloramphenicol and frusemide was carried out by high performance liquid chromatography (HPLC) as per the standard procedure. An aliquot of 10.0 μL of a standard mixture of chloramphenicol and frusemide (10.0 μg/mL of each in methanol) was injected on to the HPLC system described above. The column used was monolithic, chromolith performance RP-18e, 100–4.6 (100×4.6 mm). The mobile phase used was phosphate buffer (100 mM, pH 3.0) acetonitrile (75:25, v/v). The mobile phase was filtered and degassed before use. The flow rate of the mobile phase was 1.5 mL/min. All the experiments were carried out at $27 \pm 1^{\circ}$C. The detection was carried out at 275 nm. The chromatograms of chloramphenicol and frusemide were identified by their retention times. Chloramphenicol and frusemide in the wastewater samples were identified by comparing their retention times with those of standards. The percentage recovery of chloramphenicol into the wastewater was calculated by using frusemide. The chromatographic parameters such as retention factor (k), separation factor (α), and resolution factor (Rs) were calculated.

Quantitative Analysis

The quantitative analysis of chloramphenicol was carried out by the usual method of comparison. The quantitative estimation of chloramphenicol

and frusemide was carried out by comparing the peak areas of these drug residues in water with the peak areas of standard solutions of chloramphenicol and frusemide. The limits of detection (LOD) and limit of quantification (LOQ) were also determined by using different concentrations (1.0 to 10 μg/mL) of chloramphenicol and frusemide. The results of the statistical analysis of the experimental data such as standard deviation, correlation coefficient, and confidence levels were calculated by Microsoft Excel software program. The following equation was used to compute the concentrations of chloramphenicol and frusemide.

$$\frac{\text{Concentration of chloramphenicol}}{\text{Frusemide in wastewater sample}} = \frac{[C_{std} \times A_{samp}]}{A_{std}}$$

where,

C_{std}: Concentration of standard solution
A_{samp}: Peak area of sample
A_{std}: Peak area of standard

The percentage recovery of chloramphenicol is calculated by calibrating the method with frusemide as an internal standard. The percentage recovery of chloramphenicol is calculated by comparing the amount spiked in the wastewater and the amount obtained after extraction and HPLC analysis.

RESULTS AND DISCUSSION

High Performance Liquid Chromatography

The values of retention factor (k), separation factor (α), and resolution factor (Rs) for the separated chloramphenicol and frusemide are calculated by the standard methods. The values of retention factor (k), separation factor (α), and resolution factor (Rs) for the separated chloramphenicol and frusemide in tap and wastewater samples are given in Table 1. It is clear from Table 1 that the value of retention factors of chloramphenicol is 1.56 both in tap and wastewater, respectively. The values of separation factor (α) and resolution factor (Rs) of chloramphenicol with respect to frusemide are 2.00 and 4.30 in both tap and wastewater, respectively. All these values indicate a good separation and identification of chloramphenicol and frusemide. The chromatograms for the separated chloramphenicol and frusemide, in tap and wastewater samples are given in Figures 2 and 3, respectively. A perusal of Figures 2 and 3 clearly indicates a good separation of chloramphenicol and frusemide in tap and wastewater, respectively. The shape of the peaks are

Table 1. Retention (k), separation (α), resolution (R_s) factors and percentage recoveries of chloramphenicol and frusemide in tap and wastewater samples

Compounds	t_R	Δt	k	α	R_s	Recov. (%)
Chloramphenicol						
Tap water sample	7.35	–	1.56	–	–	95.0
Waste water sample	7.35	–	1.56	–	–	94.0
Frusemide						
Tap water sample	11.70	4.35	3.13	2.0	4.30	93.0
Waste water sample	11.65	4.30	3.13	2.0	4.30	92.0

sharp, again indicating a good separation. The chromatograms in Figures 2 and 3 have no extra peaks or noise, which confirms the proper working of the solid phase extraction method. This means that solid phase extraction is unique and specific in nature and capable to extract only chloramphenicol and frusemide from wastewater only under the reported experimental conditions.

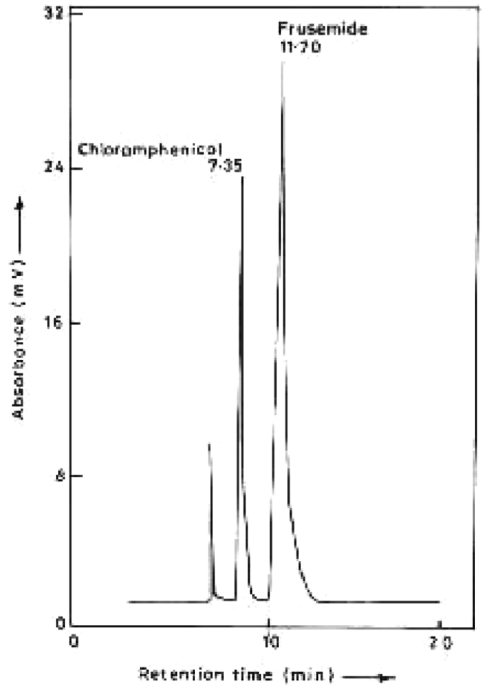


Figure 2. Chromatograms of chloramphenicol and frusemide from tap water.

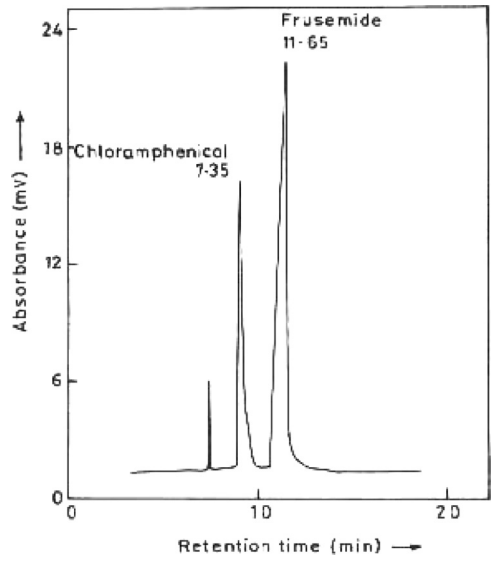


Figure 3. Chromatograms of chloramphenicol and frusemide from wastewater.

The mobile phase developed and used was phosphate buffer (100 mM, pH 3.0)–acetonitrile (75:25, v/v) with over all acidic pH of this mobile phase. Under such conditions, chloramphenicol and frusemide contain partial positive charges due to the presence of secondary amine groups. Chloramphenicol contains one secondary amine group, while frusemide shows only one secondary amine group with a sulphur atom. Besides, chloramphenicol also contains electronegative atoms (five oxygen, two chlorine, and one nitro group) while frusemide contains only five oxygen and one chlorine atom. Therefore, the positive charges are greater on frusemide in comparison to chloramphenicol. The positive charges of these molecules bind them to the $C\text{-}_{18}$ material (silonol group of silica gel) of the HPLC column through electrostatic forces of attractions. Due to these facts, frusemide is bounded strongly to the column material in comparison to chloramphenicol. Therefore, chloramphenicol eluted first followed by frusemide. Besides, the dispersion forces, hydrogen bonding, van der Waal forces, and steric effects are also playing some role in the separation phenomenon of the reported compounds.

Optimization

To optimize the separation and identification of chloramphenicol and frusemide, high performance liquid chromatographic conditions were

varied. Various solvents such as ethanol, hexane, ethylacetate, etc. were tried with phosphate buffer in various suitable combinations. Other buffers such as TRIS, acetate, borate, etc. were also tested as pure with different concentrations and pHs. These buffers were also mixed with other organic solvents such as methanol, ethanol, and acetonitrile in different combinations, and were used as the mobile phases for the separation and identification of chloramphenicol and frusemide molecules. The use of acids such as acetic acid, trifluoroacetic acid, etc. were also used in combinations of different solvents. Triethylamine, diethylamine, trimethylamine, dimethylamine, etc. were also used to optimize the chromatographic separations. After an extensive experimentation, the best solvent system developed and used was phosphate buffer (100 mM, pH 3.0)–acetonitrile (75:25, v/v).

Effect of Acetonitrile Concentrations on the Retention Times

The different percentages of acetonitrile were used to optimize the separations in the above mentioned mobile phase. The results of these findings are shown in Figure 4, which clearly indicates the effect of acetonitrile on

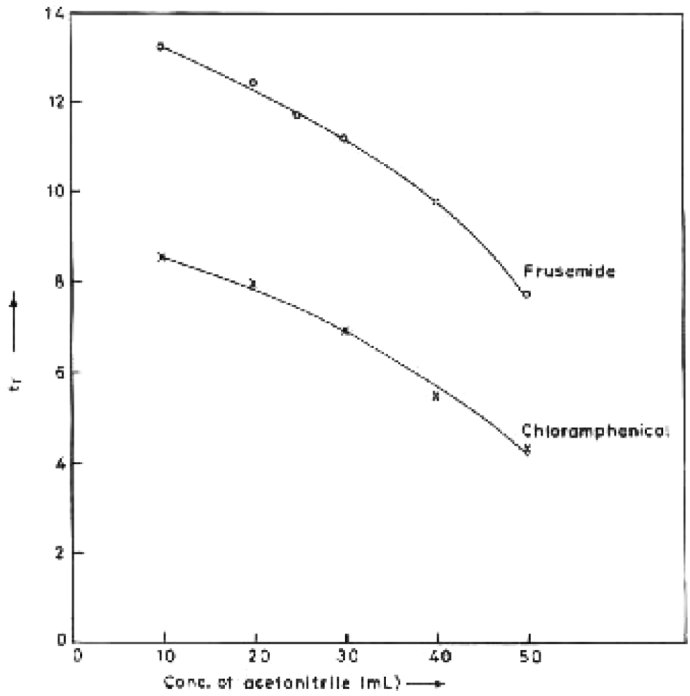


Figure 4. Effect of acetonitrile on t_r values of chloramphenicol and frusemide.

the retention times (t_r) of chloramphenicol and frusemide. It is clear from Figure 4, that t_r values decreased by increasing the percentage of acetonitrile (5 to 50%). It is also evident from this figure that the value of retention times difference (Δt) of chloramphenicol and frusemide decreased by increasing the percentage of acetonitrile indicating a poor resolution. Moreover, the peaks were broad at the low value of acetonitrile (5 to 15%) again showing an incomplete separation of chloramphenicol and frusemide. Therefore, the best mobile phase developed and used was found to be phosphate buffer (100 mM, 3.0 pH)–acetonitrile (75:25, v/v).

Effect of Flow Rate of the Mobile Phase on the Retention Times

The purpose for monolithic columns were to give fast separations. Therefore, to make the developed system fast and economic, attempts have been made to optimize high performance liquid chromatographic conditions by controlling the flow rate of the mobile phase. Various flow rates of the mobile phases tested were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 mL/minute. The effect of flow rate on the retention times of chloramphenicol and frusemide are shown in Figure 5. It is clear from this Figure that the retention times of chloramphenicol and frusemide decreases rapidly by increasing the flow rate of the mobile phase. The difference of the retention time (Δt) of chloramphenicol and frusemide also decreases at a high flow rate, indicating a poor separation of chloramphenicol and frusemide. It may be observed from this figure, that the flow rate from 0.5 to 5.0 mL/minute can be used for the satisfactory separation and identification of chloramphenicol and frusemide. However, we used 0.5 mL/min flow rate in this study.

Solid Phase Extraction

The recoveries of chloramphenicol from tap and wastewater samples were 95.0 and 94.0%, respectively, indicating good efficiency of the solid phase extraction method. Similarly, the percentage recoveries of frusemide from tap and wastewater were 93.0 and 92.0%, respectively, indicating again good efficiency of the solid phase extraction process. Slightly lower values of recoveries in the wastewater may be due to the presence of other impurities in the wastewater. No other peaks were observed on the HPLC chromatogram showing good selectivity of the solid phase extraction process. Solid phase extraction was optimized by using different eluting solvents (ethanol, ethylacetate, acetone, diethylether, chloroform, hexane, and dichloromethane), pH of wastewater, flow of eluting solvents, and other factors. As a result of extensive experimentation,

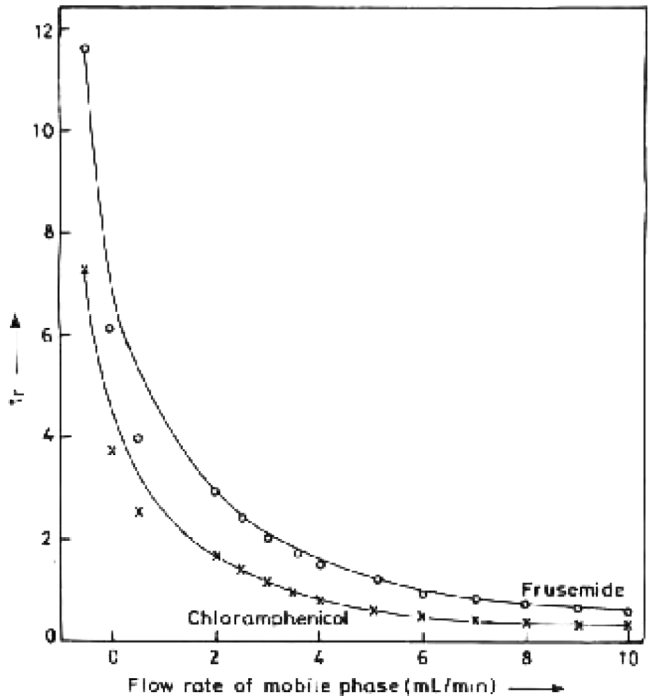


Figure 5. Effect of mobile phase flow rate on t_r values of chloramphenicol and frusemide in wastewater.

the optimized solid phase extraction conditions were developed and reported herein.

Optimization

As in the case of high performance liquid chromatography, optimization is also an integral part of the reproducible analysis in solid phase extraction. The percentage recoveries of the compounds to be extracted depend on the proper optimization of the extraction method. In view of this, attempts have been made to optimize the solid phase extraction procedure by varying the different controlling parameters for the maximum recoveries of chloramphenicol and frusemide from wastewater. The important factors that govern the recoveries of chloramphenicol and frusemide from wastewater are pH of the wastewater, flow rate of wastewater, flow rate of eluting solvent, and the use of different solvents as the eluting medium. The effect of these factors on the percentage recoveries of chloramphenicol and frusemide from wastewater are discussed herein.

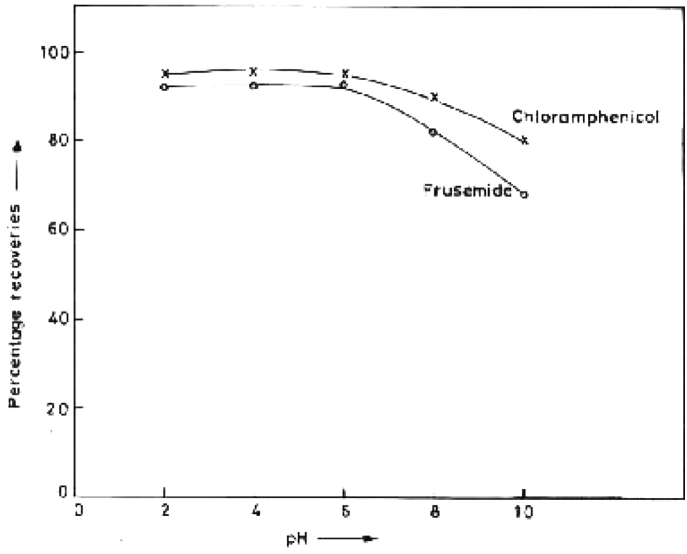


Figure 6. Effect of pH on percentage recoveries of chloramphenicol and frusemide in wastewater.

Effect of PH of the Wastewater

The ionic nature of any molecule (important for the interaction with SPE material) depends on pH of the medium and, therefore, pH of the wastewater has a major role in the percentage recoveries of chloramphenicol and frusemide. Basically, the cartridge of solid phase material is made of C_{-18} material and the percentage recoveries of the compounds depend on the adsorption process, which is controlled, up to a good extent, by the pH of the wastewater. To see the effect of pH on the percentage recoveries of chloramphenicol and frusemide, solid phase extraction experiments were carried out in the pH range of 2.0 to 10.0.

The structures of chloramphenicol and frusemide are given in Figure 1, which clearly indicates the presence of various groups as discussed above, and, hence, the positive charges are greater on frusemide in comparison to chloramphenicol under acidic conditions. The effects of pH on the percentage recoveries of chloramphenicol and frusemide are given in Figure 6, which clearly indicates that high percentage recoveries of chloramphenicol and frusemide were obtained at low pH values, while the percentage recoveries decreased at high pH values. The percentage recoveries are almost similar at 2.0 to 6.0 pH (94.0% and 92.0% for chloramphenicol and frusemide, respectively) and then decrease (up to 80.0% and 67.0% for chloramphenicol and frusemide) at high pH values

(7.0 to 10.0). This sort of behavior can be explained on the basis of the interactions between chloramphenicol and frusemide and C_{-18} material of solid phase cartridge. Under acidic conditions, both the molecules have positive charges due to the presence of secondary and tertiary amine groups. The positive charges are greater on frusemide in comparison to chloramphenicol and, hence, frusemide retains more in comparison to chloramphenicol and, therefore, frusemide has a slightly lower value of percentage recovery. The positive charges of these molecules bind (adsorption) them to the C_{-18} material (silonol group of silica gel) of solid phase cartridge through electrostatic forces of attraction. Due to these facts, chloramphenicol and frusemide are bounded to the cartridge. On elution by methanol, the percentage recoveries were found to be 94.0% and 92.0% for chloramphenicol and frusemide, respectively. As indicated from Figure 6, the percentage recoveries were almost similar at pH 2.0 to 6.0, and, hence, 6.0 pH was used through out all the experiments as it is easy to work at this pH in comparison to other low values of pHs. In this way, it may be concluded that pH of the wastewater is an important controlling factor for the maximum percentage recoveries of chloramphenicol and frusemide.

Effect of Flow Rate of the Wastewater

The maximum recoveries of any analyte can be achieved by controlling the flow rate of the wastewater through a C_{-18} cartridge. Normally, a high flow rate results in poor percentage recoveries while low flow rate is used for high percentage recoveries. Due to these points, efforts were made to optimize percentage recoveries of chloramphenicol and frusemide by controlling the flow rate of spiked water through a C_{-18} cartridge. Flow rates of 10, 25, 50, 75, and 100 mL/min were used to optimize the percentage recoveries in these experiments. The results of these findings are shown in Figure 7. It is clear from this figure that the maximum percentage recoveries were obtained at 10 mL/min (98.0 percent for chloramphenicol and 95.0 percent for frusemide), while lower percentage recoveries of chloramphenicol and frusemide were observed at 100 mL/min. The percentage recoveries of chloramphenicol and frusemide, with respect to flow rates, were in the order of $10 > 25 > 50 > 75 > 100$ mL/min. The percentage recoveries were poor when using 75 (90.0% and 80.0% for chloramphenicol and frusemide) and 100 mL/min (70.0% and 55.0% for chloramphenicol and frusemide) flow rates and, therefore, these flow rates were discarded. On the other hand, the percentage recoveries of chloramphenicol and frusemide were quite good by using 10, 25, and 50 mL/min flow rates. Hence, it was observed that the time consumed was quite high when using 10 and 25 mL/min flow rates. Moreover, the percentage recoveries were almost similar at

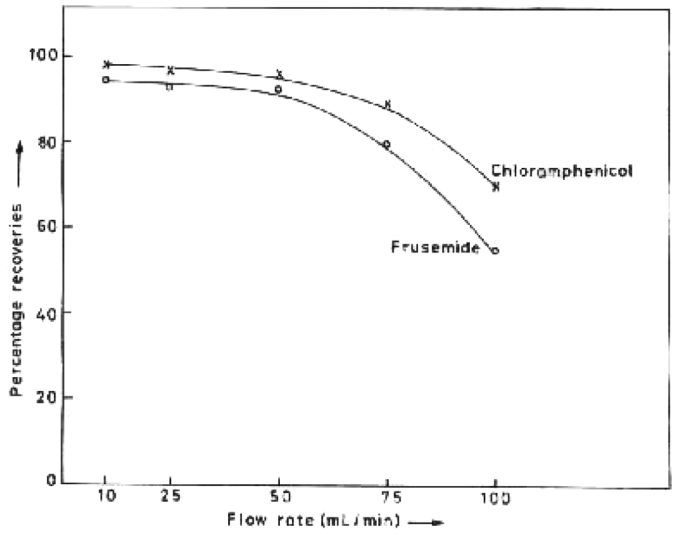


Figure 7. Effect of flow rate of wastewater on the percentage recoveries of chloramphenicol and frusemide.

all the three flow rates, i.e., 10, 25, and 50 mL/min. Therefore, the 50 mL/min flow rate was selected as the optimum one and used through out the whole experiment of solid phase extraction for the extraction of chloramphenicol and frusemide from wastewater samples.

Effect of Flow Rate of Eluting Solvent

As in the case of the flow rate of the wastewater, maximum percentage recoveries of chloramphenicol and frusemide can also be obtained by optimizing the flow of the eluting solvent (methanol) through a $C\text{-}_{18}$ cartridge. Generally, high flow rate results in poor percentage recoveries while low flow rate is used for high percentage recoveries. In view of these facts, attempts have been made to optimize the percentage recoveries of chloramphenicol and frusemide by controlling the flow rate of the eluting solvent, i.e., methanol, through the $C\text{-}_{18}$ cartridge. Four flow rates, i.e., 0.2, 0.5, 0.8, and 1.0 mL/minute were tried for maximum extraction of chloramphenicol and frusemide. The results of this set of experiments are shown in Figure 8. It is clear from this figure that maximum percentage recoveries were obtained at 0.1 mL/min, while lower percentage recoveries of chloramphenicol and frusemide were observed at 1.0 mL/min. The percentage recoveries of chloramphenicol and frusemide, with respect to flow rates, were in the order of $0.1 > 0.2 > 0.5 > 0.8$

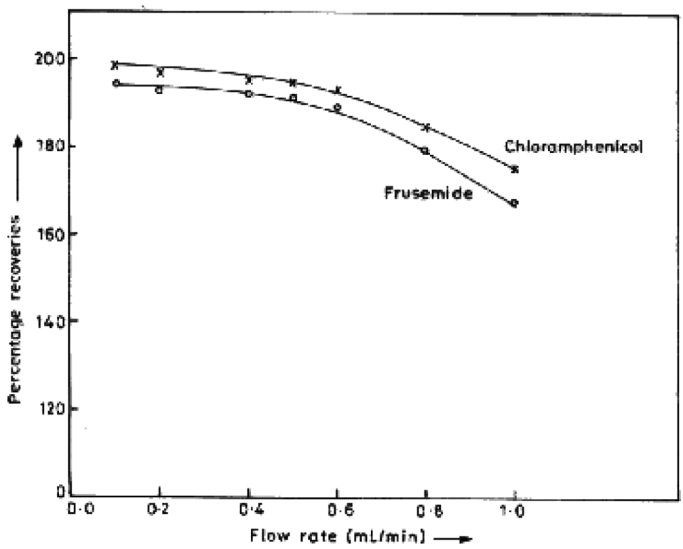


Figure 8. Effect of flow rate of eluting medium (methanol) on the percentage recoveries of chloramphenicol and frusemide from wastewater.

>1.0 mL/min. The percentage recoveries were very poor when using 0.8 and 1.0 mL/min flow rates (85.0 to 75.0 percent) and, therefore, these flow rates were not satisfactory for the extraction of chloramphenicol and frusemide. On the other hand, the percentage recoveries of chloramphenicol and frusemide were quite good by using 0.2 and 0.5 mL/min flow rates. But, t was observed that the time consumed was quite high when using a 2 mL/min flow rate. Moreover, the percentage recoveries were almost the same at 0.2 and 0.5 mL/min flow rates. Therefore, 0.5 mL/min flow rate was selected as the optimum one and used through out the whole experiment of solid phase extraction for the extraction of chloramphenicol and frusemide. Actually, the elution of chloramphenicol and frusemide from $C\text{-}_{18}$ cartridge material occurs due to the breakage of the bond (desorption) between chloramphenicol and frusemide and $C\text{-}_{18}$ material. Therefore, the low flow rate provides the maximum time to pass through the solid phase extraction resulting into maximum breakage of the bonds between chloramphenicol and frusemide and $C\text{-}_{18}$ material. On the contrary, the high flow rate does not give sufficient time to break the bond between chloramphenicol and frusemide and $C\text{-}_{18}$ material, which resulted into poor percentage recoveries of chloramphenicol and frusemide from wastewater.

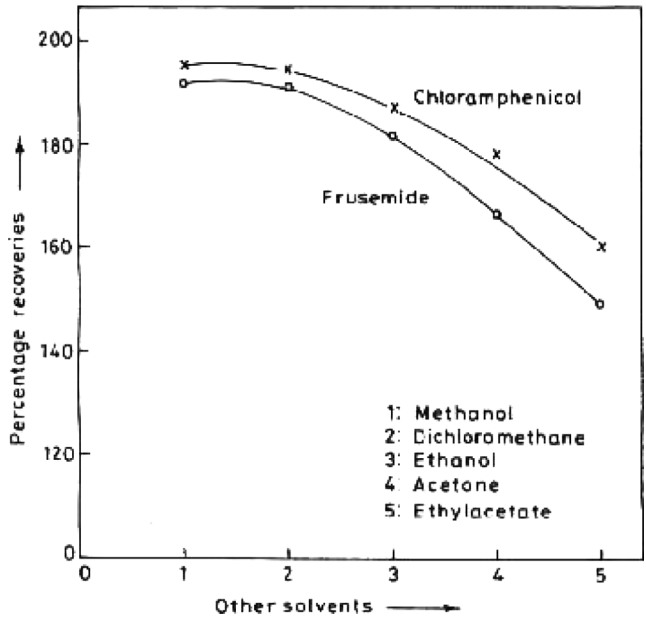


Figure 9. Effect of other solvents on the percentage recoveries of chloramphenicol and frusemide from wastewater.

Effect of Other Solvents

The optimization of solid phase extraction of any molecule requires the use of the optimum eluting solvent. Hence, various solvents were used as the eluting medium to achieve the maximum percentage recoveries of chloramphenicol and frusemide. The different five solvents used to achieve maximum percentage recoveries of chloramphenicol and frusemide include methanol, dichloromethane, ethanol, acetone, and ethylacetate. The percentage recoveries of chloramphenicol and frusemide by using these five >solvents are indicated in Figure 9. It is clear from this Figure, that the order of percentage recoveries of chloramphenicol and frusemide is methanol > dichloromethane > ethanol > acetone > ethylacetate. Maximum percentage recoveries were obtained by using methanol as the eluting medium, while the minimum percentage recoveries were obtained when ethylacetate was used as the eluting solvent. Therefore, methanol was found to be the best solvent for the elution of chloramphenicol and frusemide from a C_{-18} cartridge. This sort of behavior of these solvents may be explained on the basis of their polarity and

dielectric constants. Methanol has satisfactory values of polarity and dielectric constant and, hence, capable to break the bonds between C_{-18} cartridge material and chloramphenicol and frusemide. Dichloromethane also has good values of polarity and dielectric constant and, therefore, resulted in good percentage recoveries of chloramphenicol and frusemide as in the case of methanol. But, dichloromethane is more volatile than methanol and creates problems during experiments, especially in the concentration of the eluted solvents. On the other hand, the values of the polarities and the dielectric constants of ethanol, acetone, and ethylacetate are not quite enough to break the bonds of chloramphenicol and frusemide with a C_{-18} cartridge. Therefore, the percentage recoveries of chloramphenicol and frusemide by using these solvents are very poor.

Validation of the Methods

The validation of solid phase extraction (SPE) and high performance liquid chromatography (HPLC) methodologies was confirmed by carrying out these experiments three times ($n = 3$) under the identical experimental conditions. The regression analysis was carried out by using the Microsoft Excel program. The values of standard deviations obtained were ± 0.13 to ± 0.15 and ± 0.18 to $\pm$ 0.20 for HPLC and SPE methods, respectively. The values of the correlation coefficients (R^2) were 0.9999 to 0.9999 for HPLC and SPE methods, respectively. Further, for both methods, the confidence levels were 98.0 to 99.0%. These values of validation parameters indicate good reproducibilities of the SPE and HPLC methodologies employed.

CONCLUSIONS

The reported solid phase extraction and high performance liquid chromatographic methods were developed by carrying out extensive experimentation after optimization in chromatographic and extraction conditions. These rapid, selective, and reproducible methods were successfully used for the separation, identification, and quantification of chloramphenicol from wastewater employing a monolithic silica column. The percentage extraction of chloramphenicol is quite good. Therefore, these methods can be used for the analysis of chloramphenicol in a variety of water samples. Moreover, the developed methods may be applied to monitor the other drugs in wastewater samples of varying origin.

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