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SIMULTANEOUS DETERMINATION OF ISONIAZID AN DRIFAMPICIN BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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ABSTRACT:
In the current study we developed a micellar electrokinetic capillary chromatography method for the simultaneous determination of the most frequently used tuberculostatics isoniazid and rifampicin. The influence of pH, buffer concentration, surfactant concentrations, applied voltage, system temperature, injection parameters over the separation were studied as experimental variables. The optimal separation was carried out at 20°C and 25 kV, using a 25 mM borate buffer and 50 mM sodium dodecyl sulfate SDS at pH of 9.30. Under these conditions, the analysis was accomplished in about 3 minutes, the order of migration being isoniazid followed by rifampicin.
Keywords: isoniazid, rifampicin, tuberculostatics, capillary electrophoresis, micellar electrokinetic chromatography

INTRODUCTION:

Tuberculosis (TB) is an infectious disease caused by the bacterium Mycobacterium tuberculosis, which usually affect the lungs but can affect also other organs. TB occurs in every part of the world, but the largest numbers of new TB cases are signaled in the South-East Asia and Africa, as TB remains a disease of poverty that is inextricably associated with overcrowding and undernutrition; but today TB is curable and preventable (Gradmann, 2006).

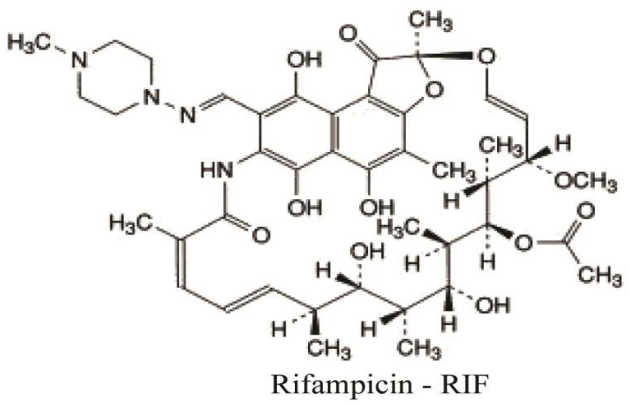
There are more than twenty drugs that are currently used for the treatment of TB, but what is alarming is that almost all of them were developed some years ago. The drugs are used in differing combinations in different circumstances. Drug-resistant TB is a serious public health issue, and is often associated with inadequate treatment protocols, lack of compliance or using low-quality medication (Caminero et al., 2010).

Of the approved drugs, isoniazid (INH) and rifampicin (RIF) are probably the most efficient and the most frequently used in combinations for the treatment of TB, being considered first-line anti-TB drugs and form the core of standard treatment regimens (Somasundaram et al., 2014).

Isoniazid (INH), is a hydrazide of isonicotinic acid, which functions by blocking the production of mycolic acid, an essential cell wall component in Mycobacterium tuberculosis; it is a prodrug activated via a bacterial catalase-peroxidase enzyme (Somasundaram et al., 2014).

Rifampicin (RIF) is a broad-spectrum antibiotic, inhibits bacterial RNA synthesis by binding to the β subunit of DNA-dependent RNA polymerase, thus blocking RNA transcription (Somasundaram et al., 2014)

The chemical structures of the two drugs are presented in **Figure 1**.



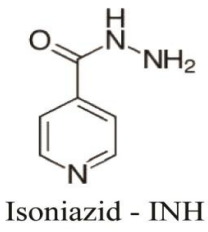


Fig. 1 Chemical structures of INH and RIF

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Taking in consideration the great prevalence of INH and RIF use in modern treatment protocols of TB, the development of new analytical methods for the simultaneous determination of the two analytes is a necessity but also a challenge for the analysts.

Literature survey revealed that several methods can be used for the simultaneous determination of INH and RIF, consisting of UV spectrophotometric (Benetton et al., 1998; Goicoechea et al., 1999; Sadeghi et al., 2006; Barsoum et al., 2008; Stets et al., 2013, Shah et al., 2014), voltammetric (Hammam et al., 2004) or RP-HPLC (Shah et al., 2014) methods.

Capillary electrophoresis (CE) encompasses a family of electrodriven techniques with distinct separation mechanisms and selectivities, which has gained ground and momentum in the analysis of pharmaceutical substances being considered today as an alternative and a complementary method to the more frequently used chromatographic techniques (Suntornsuk, 2007).

Capillary zone electrophoresis (CZE) with direct UV detection has been used for simultaneous analysis of ethambutol, isoniazid, rifampicin and pyrazinamide in pharmaceutical formulations using a complex buffer system consisting of 50 mM of acetic acid/sodium acetate buffer, 12.5 mM of $CuSO_4$ and standard and sample solutions prepared in 2 mM Brij 35 and 12.5 mM $CuSO_4$ (Faria et al., 2010).

Micellar electrokinetic capillary chromatography (MEKC) with UV detection has been described for the determination of isoniazid, pyrazinamide and rifampicin in pharmaceutical products using a buffer containing 40 mM borate buffer and 100 mM sodium dodecylsulphate adjusted to pH 8.5 (Acedo-Valenzuela et al., 2002)

The objective of this work is the development of a new alternative CE analytical method to be applied for quantitative determination of INH and RIF in combinations.

MATERIALS AND METHODS:

Chemicals and Reagents

Pharmaceutical grade Isoniazid (Merck, Germany) and Rifampicin (Antibiotice Iași, Romania) were used in the experiment. All reagents were of analytical grade quality: phosphoric acid (Chimopar Bucharest, Romania), methanol, sodium hydroxide (LachNer, Czech Republic), sodium tetraborate, disodium hydrogenophosphate, sodium didydrogenophosphate, sodium dodecyl sulfate - SDS (Merck, Germany). Deionised water was produced using a Milli-Q system (Millipore, USA).

Apparatus

Determinations were conducted on Agilent 1600 CE (Germany) system equipped with diode-array detector (DAD). Uncoated fused-silica capillary 38.5 cm length (30 cm effective length) x 50 µm ID (Agilent, Germany) was used in the separations. Electropherograms were recorded and processed by use of Chemstation 7.01 software (Agilent, Germany). Buffer solution pH was determined with a Terminal 740 pH-meter (Inolab, Germany).

Sample preparations

Sample stock solutions were prepared by dissolving the analytes in methanol in a concentration of 1 mg/mL and later diluted with the same solvent to the appropriate concentration. All samples and buffers were filtered through a 0.45 µm syringe filter and sonicated for 5 minutes before use. The samples were introduced in the system at the anodic end of the capillary by hydrodynamic injection.

Electrophoretic conditions

New capillaries were conditioned by flushing with 0.1N NaOH for 30 minutes, 10 minutes with water and 10 minutes with buffer electrolyte. Between injections, the capillary was preconditioned with 0.1 N NaOH, and then purified water, followed by the buffer electrolye, each for 2 minutes, between the runs.

In the preliminary analysis we applied some "standard" electrophoretic conditions for a CE analysis: temperature 25˚C, appliedvoltage+ 20 kV, injection pressure/time 50 mbar/1 sec, sample concentration 100 µg/mL.

RESULTS AND DISCUSSION:

Preliminary analysis

The first step in the development of the CE method for the simultaneous determination of INH and RIF was the selection of the optimal buffer pH, which determines the extent of ionization and mobility of each analyte. In order to characterize the electrophoretic behavior of the two analytes preliminary determinations were made using different buffer compositions (phosphate, borate) over a pH range between 2.5 – 11.0.

RIF is a zwitterion and has two pKa values, 1.7 related to the hydroxy group and 7,9 related to 3-piperazine nitrogen (Stets et al., 2013) and consequently is ionized over a large pH range and can be detected over the entire studied pH range.

INH has three pKa values, 1.8 based on hydrazine nitrogen, 3.5 based on pyridine nitrogen and 10.8 based on acidic group (Stets et al., 2013), has very low or even no own electrophoretic mobility and will migrate together with the electroosmotic flow (EOF) at a pH above 5.

In an acidic medium the migration times of INH and RIF were very high, above 10 minutes; while in neutral and basic environment INH migrated together with the EOF. Consequently capillary zone electrophoresis (CZE), where separation is based on the differences between the electrophoretic mobilities of the analytes, is not an ideal alternative. An electropherogram of the simultaneous determination of INH and RIF by CZE at pH 9.30 is presented in **Figure 2.**

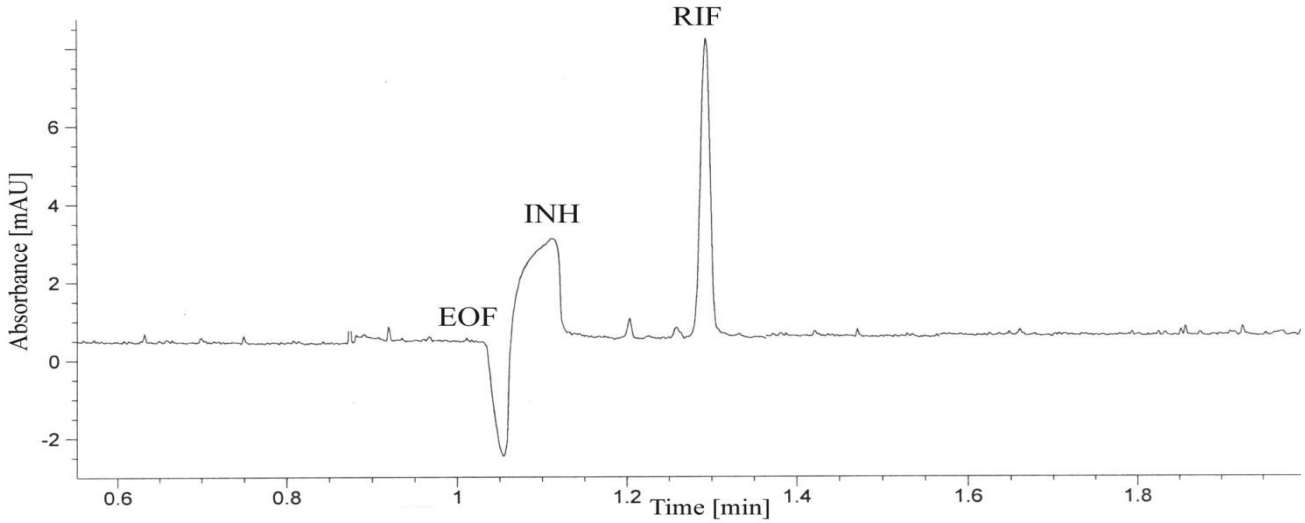

Fig. 2 CZE separation of INH and RIF (analytical conditions: 25 mM borate buffer, pH 9.30, voltage + 20 kV, temperature 25˚C, hydrodinamic injection 50 mbar/1 sec, UV detection 230 nm, sample concentration 10 µg/mL)

Taking in consideration the aspects presented above the right choice for the simultaneous determination of the two analytes is the use of a micellar electrokinetic capillary chromatography (MEKC) method, when an anionic surfactant, sodium dodecyl sulfate (SDS) is added to the background electrolyte (BGE) at a concentration higher than its critical micellar concentration (CMC). SDS was added to the buffer solution at different pH values between 8.0 and 10.0, the best results were obtained at pH 9.30. The addition of SDS in a concentration above 50 mM, separated INH from the EOF, consequently the MEKC was elected for further optimization.

Optimization of the analytical conditions

The effect of BGE concentration was studied by varying borax concentration from 25 to 50 mM at a constant pH of 9.30; an increase in buffer concentration generated high currents, indicating that more Joule heat was created; a concentration of 25 mM borax was chosen.

The effects of different concentrations of SDS was studied between 25 and 100 mM; increasing SDS concentration resulted in a more efficient separation but at the same time, raised the current in the capillary and increased migration times; a concentration of 50 mM SDS was chosen.

The effect of applied voltage was investigated over the range 15 – 30 kV; increasing the voltage resulted in shorter migration times and sharper peaks; a voltage of 25 kV was chosen.

The influence of temperature was studied in an interval between 15 – 25 C; the migration times of the analytes decreased with increasing temperature; a temperature of 20 C was selected.

As the injection times increased (1-5 sec), peak areas increased accordingly, but in the same time peak broadening was observed with injection times above 5 seconds; a injection pressure of 50 mbar and injection time of 1 second was selected.

Figure 3 shows the separation of INH and RIF by MEKC under optimized conditions.

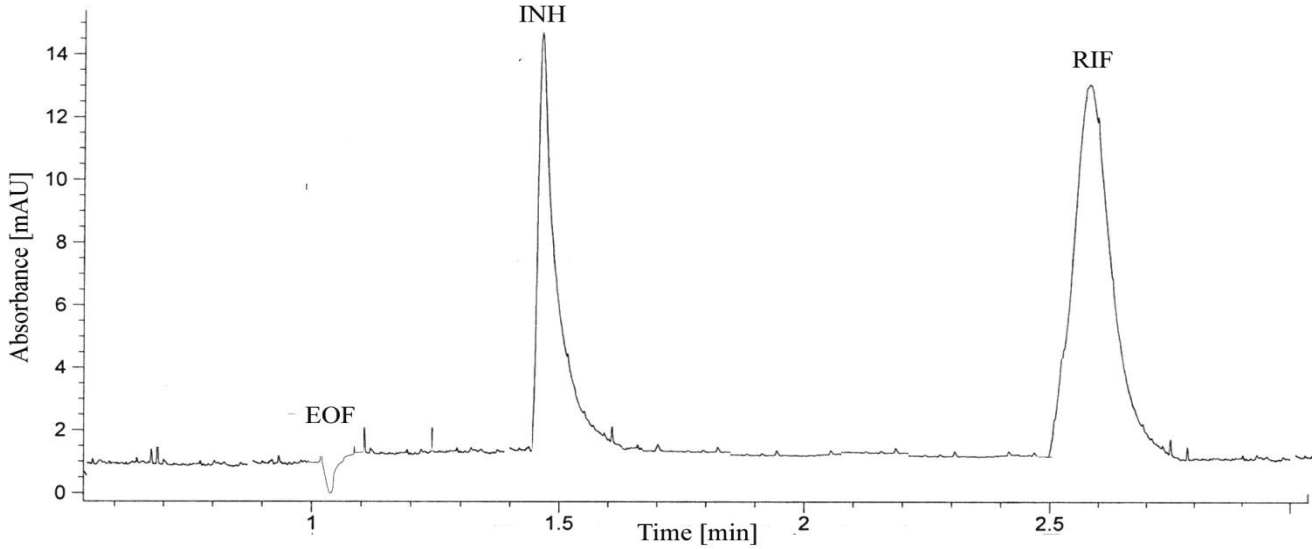

Fig. 3 MEKC determination of INH and RIF by MEKC (analytical conditions: 25 mM borate buffer + 25 mM SDS, pH 9.30, voltage + 25 kV, temperature 20˚C, hydrodinamic injection 50 mbar/1 sec, UV detection 230 nm, sample concentration 20 µg/mL)

Analytical performance

Linearity was assessed by injecting standard solutions of INH and RIF in the concentration range 5 - 100 µg/mL at six concentration levels and three replicates per concentration in the optimized separation conditions (**table 1**).

Table 1

Linearity and sensitivity data of the INH and RIF separation

Substance	Regression equation	Correlation coefficient	LOD (µg/mL)	LOQ (µg/mL)
INH	y = 0.017x – 0.0405	0.995	4.59	15.32
RIF	y = 0.013x – 0.0835	0.997	4.47	14.91

The satisfactory correlation coefficient values showed that INH and RIF responses are linear in the studied concentration range.

LOD and LOQ were estimated as (standard deviation of regression equation)/(slope of the regression equation) × 3.3 and 10, respectively (**Table 1**).

Repeatability was evaluated by performing six successive injections of two analytes in a concentration of 25 µg/mL. The reproducibility was assessed over three days by performing six successive injections each day. **Table 2** shows the most characteristic statistical data obtained with the optimal experimental conditions.

Table 2

Precision for the migration time, peak areas and peak heights of the INH and RIF separation

Substance	Intra-day precision (n = 6)			Inter-day precision (n = 18)		
	RSD (%) migration time	RSD (%) peak area	RSD (%) peak height	RSD (%) migration time	RSD (%) peak area	RSD (%) peak height
INH	0.02	0.63	0.34	0.04	1.45	1.23
RIF	0.04	0.45	0.28	0.06	1.06	1.02

The results show that the precision for the two analytes was satisfactory.

The applicability of the method was verified on prepared mixtures of INH and RIF in concentration corresponding to the ones used in therapy (**table 3**), in all cases good agreement between the calculated and the found values were obtained.

Table 3

Results obtained from INH : RIF mixtures

Mixtures	Declared amount (mg)		Found amount (mg)		RSD (%)	
	INH	RIF	INH	RIF	INH	RIF
1 : 1	150	150	150.5 ±2.4	150.2 ±2.5	0.75	0.60
1 : 2	150	300	153 ±2.2	299.8 ± 2.5	0.77	0.82

CONCLUSSIONS:

CE is a modern separation technique, with vast acceptance in the academic and industrial communities, with remarkable technological developments in the last twenty years.

The developed MEKC method is adequate for the simultaneous determination of INH and RIF, and may be used for the analysis of pharmaceuticals. The advantages of the CE related to its simplicity, flexibility and low cost of reagents and consumables, makes it an alternative technique to the more frequently used HPLC technique and it is suitable for the routine analysis of these compounds.

REFERENCES

Acedo-Valenzuela MI, Espinosa-Mansilla A, de la Peña AM,·Cañada-Cañada F, Determination of antitubercular drugs by micellar electrokinetic capillary chromatography (MEKC). Analytical and Bioanalytical Chemistry, 374, 432-436, 2002.

Barsoum NB, Kamel MS, Diab MMA, Spectrophotometric determination of isoniazid and rifampicin from pharmaceutical preparations and biological fluids. Research Journal of Agriculture and Biological Sciences, 4, 471-484, 2008.

Benetton SA, Kedor-Hackman ERM, Santoro MIRM, Borges VM, Visible spectrophotometric and first-derivative UV spectrophotometric determination of rifampicin and isoniazid in pharmaceutical preparations. Talanta, 47, 639-643, 1998.

Caminero JA, Sotgiu G, Zumla A, Migliori GB, Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. Lancet Infectious Diseases, 10, 621-629, 2010.

Faria AF, de Souza MVN, Burns RE, de Oliveira MAL, Simultaneous determination of first-line anti-tuberculosis drugs by capillary zone electrophoresis using direct UV detection. Talanta, 82, 333-339, 2010.

Goicoechea HC, Olivieri AC, Simultaneous determination of rifampicin, isoniazid and pyrazinamide in tablet preparations by multivariate spectrophotometric calibration, Journal of Pharmaceutical and Biomedical Analysis 20, 681–686, 1999.

Gradmann C, Robert Koch and the white death: from uberculosis to tuberculin. Microbes and Infection, 8, 294-301, 2006.

Hammam E, Beltagi AM, Ghoneim MM, Voltammetric assay of rifampicin and isoniazid drugs, separately and combined in bulk, pharmaceutical formulations and human serum at a carbon paste electrode, Microchemical Journal 77, 53–62, 2004.

Sadeghi S, Karimi E, Spectrophotometric determination of rifampicin through chelate formation and charge transfer complexation in pharmaceutical preparation and biological

fluids, Chemical and Pharmaceutical Bulletin, 54, 1107-1112, 2006.

Shah U, Jansani A, UV-spectrophotometric and RP-HPLC methods for simultaneous estimation of isoniazid, rifampicin and piperine in pharmaceutical dosage forms, International Journal of Pharmacy and Pharmaceutical Sciences, 6, 274-280, 2014.

Somasundaram S, Ram A, Sankaranarayanan L, Isoniazid and Rifampicin as therapeutice regimen in the current era: a review, Journal of Tuberculosis Research, 2, 40-51, 2014.

Stets S, Tavares TM, Peralta-Zamora PG, Pessoa CA, Nagata N, Simultaneous determination of rifampicin and isoniazid in urine and pharmaceutical formulations by multivariate visible spectrophotometry, Journal of Brazilian Chemical Society, 7, 1199-1205, 2013.

Suntornsuk L, Capillary electrophoresis in pharmaceutical analysis: a survey on recent applications. Journal of Chromatographic Science, 45, 459-477, 2007.