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Stepwise injection determination of isoniazid in human urine samples coupled with generalized calibration method

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ABSTRACT

Novel simple and fully automated procedure – the stepwise injection analysis (SWIA) coupled with the generalized calibration method (GCM) – has been suggested for the automation of spectrophotometric analysis of complex sample matrices. The performance of the suggested approach is demonstrated by the SWIA spectrophotometric determination of isoniazid (INH) in human urine samples. The method is based on the determination of complex between ammonium metavanadate (AMV) and INH in the presence of citrate ions which was excreted with the patient's urine in acidic medium with subsequent UV–VIS spectrophotometric detection. The conditions of complex formation have been studied for flow procedure. In order to detect, reduce matrix interferences and exclude manual sample predilution the GCM was applied. The linear ranges were from 1 to 100 mM of INH in human urine samples, and the detection limit, calculated as $3s$ of a blank test ($n = 5$), was found to be 0.3 mM. The relative standard deviation of 5 mM of INH varied in the range 1.5–3.5% ($n = 10$). The sample throughput was 5 h^{-1}. The proposed method was successfully applied for determination of N-acetyltransferase activity in human hepatocytes. The results of SWIA determination of INH were compared with those obtained by the reference method.

1. Introduction

Tuberculosis (TB) remains a major global health problem. TB is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extrapulmonary TB). The disease can spread in the air when people who are sick with pulmonary TB are coughing. Without any treatment, TB mortality rates are high. The number of TB deaths is unacceptably large given that most are preventable.

Nowadays combination of four first-line anti-TB drug (INH, rifampicin, ethambutol and pyrazinamide) was recommended as a six-month treatment for new cases of TB. For a long time INH also has been used for preventive TB therapy among people from high-risk group as well. However, a high concentration of INH in the human body due to overdosage can lead to unwanted sequela such as epilepsy, liver function failure, and even death [1,2]. Also it should be noted that the rate at which INH is metabolized in man varies considerably from one individual to another that was demonstrated in numerous studies [3–5]. Subjects can be classified as "slow" or "rapid" inactivators of INH according to the rate at which they convert the drug into metabolites that are devoid of antituberculosis activity, and the INH inactivator status of tuberculosis patients can be of clinical importance in certain circumstances [6]. Determination of INH can be as a noninvasive method for indirect determination of N-acetyltransferase activity in human hepatocytes [7]. Therefore, the determination of INH in biological fluids is an essential and frequently requested task in clinical laboratories.

A variety of analytical techniques (Table 1) have been developed for the isoniazid determination in biological fluids, such as high-performance liquid chromatography (HPLC) [8–10], capillary electrophoresis [11,12], spectrophotometry [13–18], chemiluminescence [19–25], fluorimetry [26] and voltammetry [27]. These methods are generally laborious and time consuming. In addition, chromatographic procedures require complicated sample preparation procedures such as time consuming extraction and reextraction procedures [28] thus that make them unattractive to routine clinical analysis.

Flow analysis techniques are the well-established analytical tool for solving problems of routine analysis. The main advantages of flow analysis are the low consumption of reagents and samples, better repeatability, high sample throughput, easiness for the medium exchange after analyte accumulation, reduction of the risk of contamination during the analysis step, good precision as well as a relative low cost of

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Table 1
Comparison of the suggested method with previously reported for determination of INH.

Detection technique	Sample pretreatment	On-line sample pretreatment	Sample	Linear range	LOD	Ref.
HPLC	LLExtraction	No	Urine, blood, plasma	2–100 mg L^{-1}	0.02 mg L^{-1}	[10]
HPLC	Dilution	No	Urine, plasma	12 μM–12 mM	–	[11]
HPLC	Solid-phase extraction	No	Urine, plasma	200–800 μg L^{-1}	–	[12]
CE-CL	Dilution	No	Serum, tablets	1–200 mg L^{-1}	0.3 mg L^{-1}	[13]
ECL	Dilution	Yes	Pharmaceuticals, blood	4–80 μM	2.8 μM	[14]
SP	Dilution	No	Pharmaceuticals, urine	0.05–4.50 μg L^{-1}	0.048 μg L^{-1}	[15]
KSP	Dilution	No	Pharmaceuticals	1.37–13.71 mg L^{-1}	0.15 mg L^{-1}	[16]
SP	Dilution	No	Pharmaceuticals	0.3–3.5 mg L^{-1}	0.26 mg L^{-1}	[17]
SP	Dilution	No	Pharmaceuticals	0.1–15 mg L^{-1}	0.05 mg L^{-1}	[18]
SP	Dilution	No	Pharmaceuticals	1–12 mg L^{-1}	0.5 mg L^{-1}	[19]
SP	Dilution	No	Pharmaceuticals	2–5.6 mg L^{-1}	1 mg L^{-1}	[20]
CL	Dilution	No	Pharmaceuticals	0.07–6.5 g L^{-1}	0.025 g L^{-1}	[21]
CL	Dilution	Yes	Pharmaceuticals	0.001–1.0 mg L^{-1}	0.35 μg L^{-1}	[22]
CL	Dilution	No	Pharmaceuticals	10–1000 μg L^{-1}	2.7 μg L^{-1}	[23]
CL	Dilution	Yes	Urine	2–200 μg L^{-1}	0.7 μg L^{-1}	[24]
CL	Dilution	Yes	Pharmaceuticals	0.8–100 mM	0.42 mM	[25]
CL	Dilution	Yes	Pharmaceuticals	0.05–2 g L^{-1}	0.01 g L^{-1}	[26]
CL	Dilution	Yes	Pharmaceuticals	6–200 μg L^{-1}	3 μg L^{-1}	[27]
F	Dilution	Yes	Pharmaceuticals	–	34.3 μg L^{-1}	[28]
DPV	Dilution	No	Urine	0.5–110 mM	0.17 mM	[29]
SWIA	GCM	Yes	Urine	1–100 mM	0.3 mM	This work

HPLC — High performance liquid chromatography; CE-CL — capillary electrophoresis chemiluminescence; SP — spectrophotometry; CL — chemiluminescence; DPV — differential pulse voltammetry; KSP — kinetic spectrophotometry; F — fluorimetry; SWIA — stepwise injection analysis; GCM — generalized calibration method.

the instrumentation [29,30]. The SWIA, originally suggested by our lab [31,32] assumes the mixing of the solutions in a mixing chamber (MC) by bubbling to achieve physical and chemical equilibrium state and consequently a higher sensitivity when compared with conventional FIA or SIA approach [33].

For the spectrophotometric determination of INH selective reaction between INH and metavanadate-ions has been suggested [34]. It should be noted that this reaction is carried out rapidly in acid aqueous medium, but the complex is extremely unstable. It was found that in the presence of citrate-ions the complex between INH and metavanadate-ions is more stable. However, potential instability of this complex can still be a source of inaccuracy of analytical results. For this reason the generalized calibration method (GCM) coupled with the SWIA was applied. The GCM is conceptually based on integration of the interpolative and extrapolative calibration approaches in a single procedure and progressive dilution of calibration solutions (sample and standards) in the flow mode [35]. Allowing the analytical result to be estimated by six (and not by a single – as commonly) independent values the GCM gives a chance to diagnose the analytical system examined in terms of effects influencing accuracy. Although the GCM was previously carried out with the use of different flow systems [36–38], the SWIA technique was applied for this aim for the first time.

The purpose of this study was to develop flow system on the principles of SWIA and CDM for fully automated spectrophotometric analysis of complex sample matrices. To verify the efficiency of the proposed approach, the spectrophotometric determination of water-soluble forms of INH in urine samples was performed and applied to the determination of *N*-acetyltransferase activity in human hepatocytes.

2. Experimental

2.1. Chemicals

All chemicals were of analytical grade and double-distilled water was used for the preparation of all solutions. The INH stock solution (10 mM) was prepared by reagent (Sigma-Aldrich, Germany) dissolving in water. The working solutions of INH were prepared daily from INH stock solution. The 0.5 mM solution of ammonium metavanadate (AMV) was prepared by dissolving it in 0.1 M H_2SO_4. The 0.50 M citrate buffer with pH 2.2 was prepared by dissolving 10.5 g of citric acid in water, adding 200 mL of 0.5 M NaOH and adjusting volume to 1 L. The required pH of the citrate buffer solution was achieved by the addition of 0.5 M H_2SO_4. The 0.5 M phosphate buffer with pH 7.4 was prepared by dissolving K_2HPO_4 and KH_2PO_4 in water. AMV solution, citric buffer and phosphate buffer were stored in a dark place at 5 °C and used within 5 days. The acetonitrile was of HPLC grade (Merck, Germany). Pills of pharmaceutical – grade isoniazid (Moschempharmpreparaty, Russia) were used as model drug.

2.2. Apparatus

2.2.1. Stepwise injection set-up

For the stepwise injection procedure (Fig. 1) the PIAKON-30-1 flow analyzer (Rosanalit, Russia) was employed. This manifold includes two six-way solenoid valves (Cole-Parmer Inc., Vernon Hills, IL, USA), peristaltic pump (PP) MasterFlex L/S (Cole-Parmer Inc., Vernon Hills, IL, USA) ensuring a reverse flow (flow rate is from 0.5 to 5 mL min^{-1}), six similar mixing chambers (PTFE 10 mm of i.d. and 50 mm in height) with a funnel-shaped inlet at the bottom. The MCs were connected to the ports of the solenoid valve (1) via by PTFE tubes (0.75 mm of i.d. and 20 mm in length). The lateral ports of the solenoid valve (2) were connected with the citrate buffer (port 1), AMV solution (port 2), INH solution (port 3) and sample (port 4) by PTFE tubes. Port 6 was connected to the atmosphere to ensure the mixing of the reagent solutions and sample into the MCs. Port 6 of the solenoid valve (2) was directed to a 50 mm path-length flow cell (FIAlab® Instrument Systems Inc., Bellevue, WA, USA). The manifold was equipped with a USB 4000 spectrophotometer (Ocean Optics Inc., Winter Park, FL, USA) with optical fibers QP400-2-UV–VIS (Ocean Optics Inc., Winter Park, FL, USA) and a Model D 1000 CE UV source (Analytical Instrument System Inc., USA). The flow analyzer was operated automatically by means of a computer.

2.2.2. Reference method set-up

HPLC analysis was carried out on a Shimadzu LC-20 Prominence liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) with UV detection. The chromatographic separation was achieved by Supelco C18 HPLC column (250 × 4.6 mm, 5 μm particles size). The mobile phase used was 94% phosphate buffer (0.5 M, pH 7.4) and 6% acetonitrile at flow rate 1 mL min^{-1}. UV detection was used for INH (266 nm between 0 and 4 min). Measurements were carried out in the normal mode, the column temperature was 35 °C.