Determination of Selected Organic Acids in Animal Farm Water Samples by Ion Chromatography

Marta Wasielewska, Anna Banel, and Bogdan Zygmunt

***Abstract*—Low molecular mass carboxylic acids (LCAs) occur in many environmental compartments due to natural processes and human activity. They are an important group of organic pollutants of waste water that mainly includes, volatile fatty acids, and also dicarboxylic, aromatic, hydroxy- and keto-acids. LCAs are present in the environment at concentrations between 1 to 5000 ppm and different methods have been reported for their determination [1]. When volatile and nonvolatile acids are to be monitored then liquid chromatography, including ion chromatography is the method of choice. At high pH the acids are in ionized form and can be separated as carboxylates by means of anion exchange chromatography. LCAs can also be separated at low pH by ion exclusion chromatography or by reverse phase chromatography. Using Dionex-3000 instrument with an Acclaim Organic Acid, OA column dedicated to separation of organic acids the method has been developed to identify selected LCAs. With this analytical tool formic, acetic, propionic, butyric and also citric, malonic, and oxalic acids were measured in waste water from swine and cattle livestock farms**.

***Index Terms*—Environmental analysis, organic acids, anion chromatography, ion exclusion chromatography, reverse phase column.**

I. Introduction

The occurrence of low molecular mass carboxylic acids (LCAs) in the environment stems from a multitude of biological sources, geological processes, bio-geochemical reaction and emissions related to human activity.

LCAs are a group of organic compounds that contain one or more carboxylate functional groups (-COOH) and a short hydrocarbon group which can be aliphatic, aromatic, saturated or unsaturated, straight chain or branched, and substituted with hydroxyl- or keto-, or any other group.

Organic acids are secreted by many organisms like bacteria, fungi, algae, higher plants or animals. Many can be formed from larger biological molecules, e.g. short chain monocarboxylic acids, often referred to as volatile fatty acids (VFAs), are produced in anaerobic biodegradation of proteins, carbohydrates and fats.

The important anthropogenic sources include the residues and by-products of agriculture and food processing, waste water, organic waste, sewage sludge, landfill leachate and various technical systems and treatment and disposal facilities.

In the case of waste water, e.g. very important representatives include: alkane monocarboxylic acids with up to six carbon atoms in a molecule, and also non-volatile acids such as oxalic, succinic and pyruvic acid. In fact, LCAs occur in different environmental compartments due to natural processes as well as because of human activity.

Some play an important role in biological waste water treatment since they are a source of easily assimilated carbon for microorganisms. Some volatile acids, mainly propionic and butyric acids, are responsible for unpleasant smells from waste water treatment plants and solid waste landfills.

LCAs can increase mobility of heavy metals and radionuclides in the environment and possibly interact with metal ions and minerals. As a consequence, organic acids are ubiquitous in the ecosphere.

Therefore, individual LCAs should be monitored in many media.

Gas chromatography is the method of choice for determination of volatile fatty acids. However, often nonvolatile organic acids are also of interest and then other separation techniques such as high performance liquid chromatography or capillary electrophoresis should be applied.

Carboxylic acids undergo dissociation and if pH is sufficiently high they are mainly in ionized form and can be separated as carboxylate anions by anion exchange chromatography (AEC). When in ionic form they can also be determined by electrophoretic techniques. Ion exclusion chromatography (IEC) with cation exchange columns is applicable and increasingly often used to separate LCAs.

A. Anion Exchanged Chromatography

In ion chromatography the stationary phase carries functional groups with a fixed charge. The respective counter ions are located in the vicinity of these groups and hence the whole entity is electrically neutral. In anion exchange chromatography covalently bonded functional groups of the stationary phase carry a positive charge while negatively charged species can travel between the stationary phase and mobile phase. The latter is a liquid containing an electrolyte in a dissociated form, e.g. sodium bicarbonate, whose anions fully neutralize the fixed positive charge of the stationary phase. When a sample is introduced into the column and analyte ions are capable to replace counter ions in a reversible way, the analyte moves along a column with velocity smaller than the mobile phase. If the interaction of two substances with the stationary phase differs then they can be separated.

Due to the pioneering work of Small, Stevens and Bauman [2] on low capacity ion exchange resins high performance ion chromatography (HPIC) could have been developed. The stationary phases for high performance anion exchange

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chromatography (HPAEC) are mainly latex-based anion exchangers. They consist of surface sulphonated highly crosslinked styrene-divinylbenzene copolymer particles of several μm in diameter coated with fully aminated, high capacity latex microbeads of tens (e.g. 70, 85) of nm in diameter. Being an anion exchanger the latex microbeads are responsible for anion retention and separation. Latex is polyvinyl chloride or polymethacrylate.

The mobile phase is generally an aqueous solution of hydroxide, carbonate, bicarbonate or borate. In majority of AEC applications aqueous samples usually do not require any sample pretreatment other than filtration prior to injection. To protect the analytical column from degradation and to prolong its life time guard columns are applied in-line prior to the analytical column. Detection is generally based on conductivity measured after eluent conductivity is suppressed [3]. HPAEC with an aqueous KOH solution at a flow rate of 0.8 mL/min as a mobile phase was successfully used to determine water soluble short chain carboxylic acids (acetic, formic, propionic, glutaric, adipic, oxalic, succinic, malic, malonic, maleic) in ambient aerosols. The separation was performed on an Ionpac AS17 analytical column of 4 mm I.D. The column is based on a microporous EVB/DVB copolymer with a particle diameter of 10.5 μm and a degree of cross-linking of 55%. The latex particles have a degree of cross-linking of 6% and a diameter of 75 nm and carry strongly hydrophilic anion exchange groups. With conductivity detection (after suppressing) the acids were detected on the levels of single μg/L in aqueous extracts. The AEC applications to determine LCAs have been reviewed [4].

B. Ion Exclusion Chromatography

To determine low molecular mass carboxylic acids in aqueous samples ion exclusion chromatography (IEC) is increasingly applied [4],[5]. The introduction of the technique is attributed to Wheaton and Bauman [6]. In high performance ion exclusion chromatography (HPIEC) typical stationary phases are totally sulphonated cation exchange resins. Eluents applied are aqueous solutions of mineral and organic acids sometimes modified with organic solvents such as acetonitrile and alcohols to reduce tailing and retention times of more hydrophobic analytes. Rather complex retention mechanisms are based on such phenomena as Donnan exclusion, size exclusion, adsorption, polar interactions, hydrogen bonding, etc. whose contribution in total retention depends on the acid nature [3], [5]. Aliphatic monocarboxylic acids retention is determined by Donnan exclusion and adsorption so it increases with increasing length of alkyl chain. It will also be influenced by pH since only uncharged species can pass freely through the Donnan barrier and undergo adsorption. Apart from Donnan exclusion, steric exclusion is a predominant phenomenon in the case of di- and threecarboxylic acids, so the size of the sample molecules is the factor which makes that the substance is retained by the resin of a given pore volume dependent on cross-linking.

Typically, suppressed conductivity detection is used in HPIEC but more powerful analytical detection and identification machines are produced by coupling it with mass spectrometry [7], [8]. The technique was widely used in determination of LCAs in a variety of samples [4]. Dias et al. [7]. developed a method to determine acetic, propionic and butyric acids in dietary fiber extracts using HPIEC. The analytical column (100 x 7.8 mm) was packed with 10 μm particles of PS/DVB copolymer functionalized with sulphonic acid groups. A mobile phase was aqueous solution of sulphuric acid (0.5 mmol/L). With inverse chemical suppression and conductivity detection the quantitation limits obtained were from 5 up to 25 μmol/L.

Using a typical HPIEC column (250 x 7.8 mm, 10 μm particles), and perchloric, heptafluorobutyric and sulphuric acids at different concentrations as mobile phases and conductivity detection after inverse suppression, eleven saturated and unsaturated LCAs were separated in 22 min [9]. However, to aid quantification of maleic and oxalic acids AEC was used. The detection limits ranged from 10 to 500 ppb.

C. Ion Pair Chromatography

In the latest decade, a growing interest has been noted in the field of the determination of organic acids in biological, clinical and food samples. An attractive alternative to ion exchange and ion suppression analysis of ionic samples is the technique commonly referred to as ion-pair chromatography (IPC). IPC, same as IEC, is can be applied to separate charged analyte properties, and is frequently used to determine substances, which are polar and of low molecular mass [10].

The application of reversed-phase ion-pair chromatography (R-IPC), as compared to ion-exchange chromatography, to separate charged analytes has the advantage that both neutral and ionic species can be separated. Wang and Liao [11] studied the determination of low molecular mass organic acids of abnormal metabolic markers studied. The pH of the eluent was adjusted in order to encourage the ionization, for acids pH 7.5 was used and for bases pH 3.5. The chromatographic retention was altered by adding an ion pair reagent, a large bulky ion of the charge opposite to analyte charge; it is called counter-ion; it is present in the mobile phase and can form a neutral ion-pair with the ionic sample components.

II. Determination of Carboxylic Acids in Pig and Cattle Farms Waste Water

Generally only short chain monocarboxylic acids are monitored in waste waters and then GC is a method of choice [12], [13]. However, some nonvolatile or poorly volatile LCAs can also be present in waste water and they should be monitored. In this work an ion chromatograph equipped with a specialty column was applied to determine VFAs and other LCAs in swine and cattle farm waste water.

A. Chemical and Materials

All materials were of analytical-reagent quality (purity > 99%) unless stated otherwise. Formic acid, acetic acid, propionic acid, butyric acid (≥99,5%) and oxalic acid, citric acid, malonic acid (≥99,9%) were purchased from Fluka (Buchs, Switzerland). Methanesulphonic acid (99,5%) was

also received from Fluka. Acetonitrile was from Sigma-Aldrich. In all experiments ultrapure Mili-Q water (Milipore QPLUS185) was used.

B. Instrumentation

Chromatographic analysis was carried out using a Dionex ISC-3000 ion chromatograph equipped with a UV detector and an autosampler AS50 Auto Select. Diluted solutions of volatile fatty acids with one to four carbon atoms in a molecule as well as citric (hydroxy group), malonic and oxalic acids (dicarboxy) were separated on an Acclaim Organic Acid, OA 4.0 x 150 mm (Dionex Bonded Products) column – it is packed with 5-µm high purity spherical silica particles with 120-Å diameter pores. A proprietary functional group, selectively retaining organic acids, is bonded to the particle. The column provides unsurpassed resolution and efficiency of common hydrophilic organic acids.

C. Sample Preparation and Storage

Waste water samples were taken from pig farm and also from cattle farm. The samples were vortexed (4000 rpm) and filtered with a 0.45µm membrane filters (IC – PTFE) and stored in polyethylene containers at 4 ◦C. The samples were injected to the chromatographic system after dilution with deionized water in the proportion of 1:10.

D. Operating Conditions

A mixture of methanesulphonic acid (A) and acetonitrile (B) was used as the eluent for the determination of organic acid anions. The system was operated in a gradient mode. The aqueous methanesulphonic acid solution (2.5 mmol/L) was designated as solvent A and acetonitrile as solvent B.

To get a good separation of volatile short-chain acids and also dicarboxy- and hydroxyacids acids flow rate in gradient mode, was from 0.3 ml/min to 0.6 ml/min.

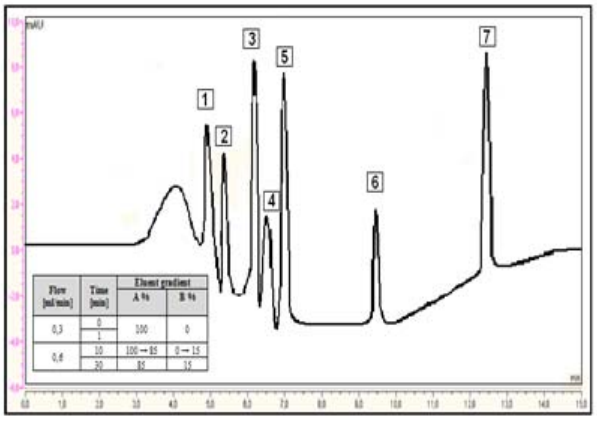

Fig. 1. The chromatogram of a standard solution of selected LCAs at concentrations of 100 mg/L each (except for oxalic acid – 25 mg/L). 1 – oxalic acid, 2 – formic acid, 3 – malonic acid, 4 – acetic acid, 5 – citric acid, 6 – propionic acid, 7 – butyric acid

E. Results

The most challenging task was to separate volatile short-chain carboxylic acids as well as dicarboxy- and hydroxyacids together in one sample. Applying an Acclaim Organic Acid, OA column and gradient elution (aqueous solution of methanesulphonic acid + acetonitrile) and optimised temperature, the separation of formic, acetic, propionic, butyric and also citric, malonic, and oxalic acids was achieved. The chromatogram of a standard solution of these seven LCAs is given in Fig. 1.

The method developed was applied to analyze swine and cattle farm waste water for the content of carboxylic acids. The concentrations of particular acids are shown in Table I and Fig. 2. The chromatogram of an exemplary sample of swine and cattle farm waste water is given in Fig. 3.

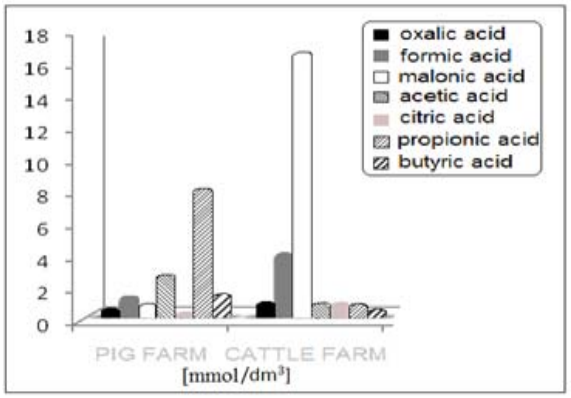

Fig. 2. Concentration of particular acids in samples collected from pig and cattle farm

Acids \ Concentration [mM/L]	Swine Farm	Cattle Farm
Formic Acid	3.9	1.22
Acetic Acid	0.80	2.54
Propionic Acid	0.73	7.91
Butyric Acid	0.39	1.34
Oxalic Acid	0.86	0.28
Malonic Acid	16.0	0.73
Citric Acid	0.83	0.20

III. Conclusion

Column chromatography (Aclaim OA, Dionex) is applicable to determine 7 organic acids such as oxalic acid, formic acid, malonic acid, acetic acid, citric acid, propionic acid and butyric acid in two types of waste water.

The separation and quantitation of the low – molecular acidic acids, monocarboxylic-, dicaroxylic- keto- and hydroxyl- can be conducted using the same chromatographic conditions within 15min (total analysis time) and a dual detection system (UV, 210 nm).

Low molecular mass carboxylic acids should be monitored in aqueous samples of different origin. This method developed can be easily applied for routine analysis of the waste water samples of different origin

The main parameters related to sensory evaluation can be determined using this approach. When volatile and nonvolatile acids are of interest liquid chromatography, especially equipped with ion exchange separation systems (anion exchange, ion exclusion) or with reverse phase specialty columns can be successfully used. Ion exclusion chromatography is increasingly used though sometimes combined with anion exchange chromatography and also reverse phase systems can be applied. Dionex ion chromatograph equipped with an Acclaim Organic Acid

specialty column can be successfully applied to analyze swine farm wastewater for the content of volatile alkane monocarboxylic and non-volatile organic acids, e.g. formic, malonic, acetic, citric, propionic, butyric, and oxalic acids.

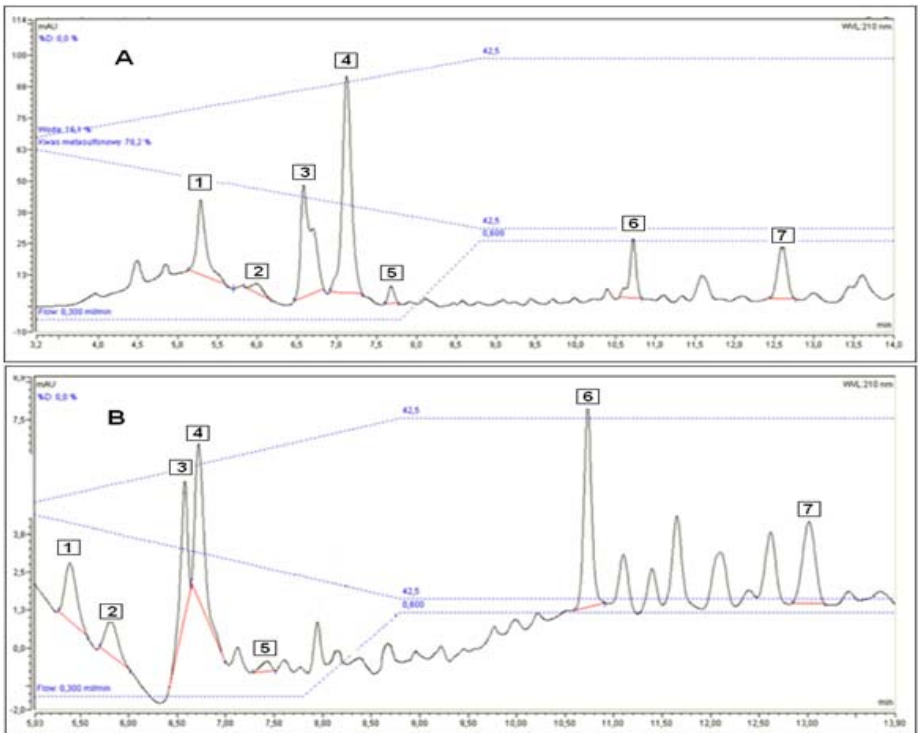

Fig. 3. Chromatogram of an exemplary real samples of swine farm (A) and cattle farm (B) waste water. 1 – oxalic acid, 2 – formic acid, 3 – malonic acid, 4 – acetic acid, 5 – citric acid, 6 – propionic acid, 7 – butyric acid

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