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HPLC determination of salinomycin and related compounds in fermentation media of *Streptomyces albus* and premixes

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Abstract

A rapid, sensitive and selective HPLC method with post-column derivatization was proposed for the determination of salinomycin and related products in fermentation broths and premixes. The solvent extracts of samples were analysed on a reversed-phase monolithic type column. The mobile phase consisted of methanol/zinc acetate (0.05 M) adjusted to pH 4.0 with acetic acid (85/15, v/v). Post-column derivatization with vanillin at 85 °C was used for simultaneous, selective detection of salinomycin at 520 nm and related products at 460 nm. Optimal ratio of mobile phase/reagent flow rate was 2:1. Alternatively, pre-column derivatization of salinomycin and related products with three different reagents (2,4-dinitrophenylhydrazine, *p*-bromophenacyl bromide and *p*-nitrobenzoyl chloride) was examined. Suitable derivates for HPLC separation and UV detection were prepared using *p*-nitrobenzoyl chloride. Extraction ability of various solvents for extracting of salinomycin and co-products from premix samples was also tested. Acetone, ethanol and pyridine were found to be the best extraction solvents for these compounds.
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Keywords: Salinomycin; Related products; Reversed-phase liquid chromatography; Fermentation broth; Premix; Solvent extraction

1. Introduction

Salinomycin (Fig. 1) belongs to a group of polyether antibiotics (ionophores), which include monensin, narasin and lasalocid.

The usual structural features include a carboxyl group at one terminus and a hydroxyl group at the other. They readily form cyclic complexes with cations, particularly alkali metals (sodium), and have the ability to cross biological membranes [1]. The main therapeutic application of the polyether antibiotics is for the prevention and treatment of coccidiosis in poultry [2].

Salinomycin is produced by a fermentation process using *Streptomyces albus* strain [3]. Along with the main product, also related compounds (co-products) of salinomycin are originated during this process [4]. These are described as salinomycin type antibiotics because they have the similar chemical structures and properties. This group includes 20-deoxy salinomycin and its streoisomers, 18,19-dihydro salinomycin and its stereoisomers and 5-hydroxy salinomycin [4]. The appearance and quantity of these compounds in fermentation media is mainly dependent on conditions of fermentation; however, 20-deoxy salinomycin ant its stereoisomer (Fig. 2) are produced in high yield [4].

Quantity of some salinomycin related compounds (17-epi-20-deoxy salinomycin) in final salinomycin product are limited [5] and therefore production of these compounds is monitored.

Microbiological as well as chemical methods have been described for determining salinomycin in premixes [6–16] and fermentation broths [9,13,15]. Recently chromatographic methods (especially HPLC) become widely used for accurate, precise, sensitive and selective quantification. Because salinomycin has no significant UV, fluorescent, or electrochemical properties, it must be derivatized either

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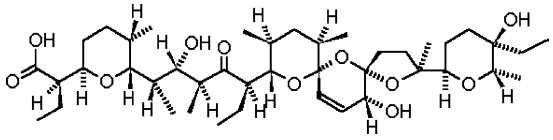

Fig. 1. The chemical structure of salinomycin.

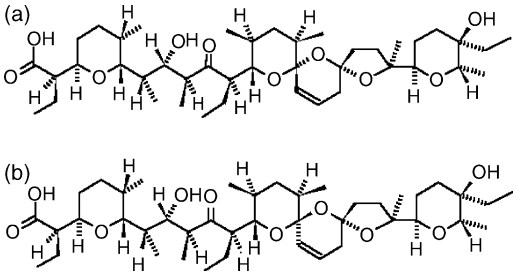

Fig. 2. The chemical structure of (a) 20-deoxy salinomycin and (b) 17-epi-20-deoxy salinomycin.

pre- [11,14,16] or post-column [8–10,12] for detection after HPLC separation. A direct UV detection of salinomycin at 210 nm was also proposed [13], but due to complexity of matrices it seems to by unsuitable for determination of salinomycin related products. Also LC–MS technique was found to be suitable for the sensitive and selective determination of residues of various ionophores in feed [17], animal products [18,19] or manure [20]. Presented papers focused on determination of salinomycin as major component and determinations of related compound were mentioned only rarely [12].

This paper deals with the development of an HPLC method suitable for the fast determination of salinomycin and related compounds in production fermentation broths and premixes. Applicability of different pre- and post-column derivatization techniques was verified with regard to their sensitivity and selectivity.

Important part of an analytical method is extraction and clean up of monitored compounds from matrix. The commonly utilized techniques for the extraction and clean up of drugs from biomatrices involve liquid–liquid extraction (LLE) or solid-phase extraction (SPE). Initial extraction of ionophores from fermentation broths or premixes has traditionally been accomplished with the use of methanol [8,14], ethanol [11,13], acetonitrile [15], or hexane [12]. Asukabe et al. [21] reported that a wide variety of solvents (methanol, acetone, ethyl acetate, acetonitrile, benzene and hexane) are comparable for extracting most ionophores. Blanchflower et al. [8] compared variety of solvents for extracting of salinomycin and other ionophores from feed samples. Best recoveries of salinomycin were reported for methanol (Recovery (R) = 100%), methanol–water mixture (90:10, v/v) (R = 96%), acetone (R = 91%) and ethanol (R = 86%). Our intent was comparison of selectivity of different solvents for extracting not only salinomycin but also co-products from premix sample.

2. Experimental

2.1. Chemicals

2,4-Dinitrophenylhydrazine, *p*-bromophenacyl bromide, *p*-nitrobenzoyl chloride and vanillin all analytical grade were obtained from Acros Organics (Geel, Belgium). HPLC grade methanol, tetrahydrofuran, acetonitrile and analytical grade ethanol, 2-propanol, 1-butanol, diethyl ether, pyridine, *N*,*N*-dimethylformamide, dichloromethane, acetone and hexane were obtained from Merck (Darmstadt, Germany). Analytical grade acetic acid, sulfuric acid, sodium acetate trihydrate, ammonium acetate, potassium acetate, zinc acetate dihydrate, lithium citrate tetrahydrate and magnesium acetate tetrahydrate were obtained from Fluka Chemie (Buchs, Switzerland). Water was deionized and distilled. Salinomycin sodium, 17-epi-20-deoxy salinomycin sodium and 20-deoxy salinomycin sodium standards were prepared in R&D department of Biotika a.s. The identity and purity of salinomycin was employed by comparison with salinomycin purchased from Sigma (Deisenhofen, Germany). The identity of 17-epi-20-deoxy salinomycin and 20-deoxy salinomycin was confirmed according to published data [4,22,23]. Due to insufficient amount of prepared 20-deoxy salinomycin standard, it was used only for qualitative confirmation.

2.2. Standard preparation

The standard solution was prepared by dissolving salinomycin or 17-epi-20-deoxy salinomycin in methanol to give a concentration of about 1 mg ml^{-1} of each substance. These solutions were stable for at least one week when stored at −20 °C.

2.3. Sample preparation

2.3.1. Fermentation broth

About 5 g of fermentation broth was weighed in to a 50 ml volumetric flask and diluted to volume with extraction solvent. The solution was sonicated for 10 min in an ultrasonic bath. The solution was filtered through a 0.45 μm membrane filter and analysed. The final concentration of salinomycin was in the range of 0.5–1.5 mg ml^{-1}.

2.3.2. Premix samples

Premix samples were pulverised in a grinder to obtain a homogeneous powder. About 0.5 g was weighed in to a 50 ml volumetric flask and diluted to volume with extraction solvent. The solution was sonicated for 10 min in an ultrasonic bath. The solution was then filtered through a 0.45 μm membrane filter and analysed. The final concentration of salinomycin was about 1 mg ml^{-1}.

2.3.3. Vanillin reagent

20.0 g of vanillin was weighed in to 500 ml volumetric flask and dissolved in about 300 ml of methanol, and then

10 ml volume of 96% sulfuric acid was slowly added with constant stirring to avoid overheating. Finally methanol was added to volume and solution was mixed. Reagent was kept in dark bottle at 5 °C.

2.4. Instrumentation

The HPLC system consisted of a Shimadzu (Kyoto, Japan) model LC-10AS single piston pump, a model SIL-10AXL auto injector, a model CTO-10AC column oven and a model SPD-10AV UV–vis detector all controlled by a computer using Class-VP 4.0 software. Separation was carried on a monolithic type HPLC column Chromolith RP-18e (100 × 4.6 mm) (Merck). The column temperature was maintained at 35 °C. The injection volume was 20 μl. Post-column reaction system consisted of a Shimadzu LC-10AS single piston pump, a low dead volume T-piece, a teflon reactor coil (10 m × 0.5 mm), and a Shimadzu model column oven CTO-6A.

3. Results and discussion

3.1. Pre-column derivatization

Suitable UV derivates of salinomycin and its co-products could be prepared utilizing reactions with keto, carboxyl or hydroxyl groups of these compounds. Derivatization of the keto group of salinomycin with 2,4-dinitrophenylhydrazine under acidic conditions forms a highly UV-active hydrazone, which can be separated on HPLC column [11,16]. Under described reaction conditions [16] similar results were obtained for salinomycin, but 17-epi-20-deoxy salinomycin was unstable and many degradation products (peaks) were observed on HPLC chromatogram after derivatization. Reaction mixture was analysed on Chromolith RP-18e (100 × 4.6 mm) column using methanol–formic acid (0.1 vol.%) (85:15, v/v) as mobile phase. Flow rate was maintained at 2.0 ml min^{-1} and detection was at 395 nm.

p-Bromophenacyl bromide was used to derivatize the carboxyl group of salinomycin and 17-epi-20-deoxy salinomycin according to conditions described by Korte et al. [24]. On both chromatograms of reaction mixtures a major peak with the same elution time was presented, that indicates formation of derivates with similar retention properties. Separation conditions were identical as for separation of hydrazones except for the detection, which was at 245 nm.

Better results were achieved by derivatisation of hydroxyl groups with *p*-nitrobenzoyl chloride applying reaction conditions according to Gullo et al. [25]. In this case reaction of both salinomycin and 17-epi-20-deoxy salinomycin was rapid (5–10 min at laboratory temperature) and peaks of both derivates were completely separated. Reaction mixture was analysed applying same separation conditions, as for separation of hydrazones except for the detection, which was at 260 nm. The major drawback of this method was quite difficult sample pre-treatment due to necessity for anhydrous and "hydroxyl free" reaction mixture.

However, pre-column derivatization techniques were considered as appropriate for the determination of main component (salinomycin) [11], determination of salinomycin related compounds seems to be problematic due to instability of derivates, interference of reagents, complicated sample pre-treatment or formation of unexpected reaction by-products. In addition pre-column reactions modify the structure of target analytes and thus their retention properties, which can cause co-elution of target compounds. These disadvantages could be eliminating using derivatization after column separation, as is stated thereinafter.

3.2. Post-column derivatization

Post-column derivatization of polyether antibiotics usually employs reaction with vanillin reagent followed by detection at 520 nm [8,9,12]. Although 4-dimethylaminobenzaldehyde has been reported as a more sensitive derivatizing reagent for the detection of salinomycin and narasin [10], vanillin has been widely used as reliable reagent for detection of broad range of polyether antibiotic in different matrices. Prior to derivatization polyether antibiotics are generally separated on particulate packed reverse phase type columns [8–10]. Initial conditions of our separation system were proposed according to experiences regarding the determination of salinomycin with direct UV detection [13]. The particular column was substituted with monolithic type column, which has benefit of lower operating pressure and higher throughput and therefore is ideal for rapid analysis. Mobile phase consisted of 15 volumes of 0.2 M sodium acetate buffer (pH = 5.8) and 85 volumes of methanol. Flow rate of mobile phase was maintained at 1 ml min^{-1} and flow rate of vanillin reagent at 0.5 ml min^{-1}. During the first experiments we had occasional problems with clogging of reaction coil, and therefore concentration of buffer in mobile phase was decreased from 0.2 to 0.05 M. Under the suggested conditions good separation was achieved; however, some peaks were unresolved and only salinomycin had good response at 520 nm. Therefore, selectivity of the separation conditions and increasing of response of these compounds was further optimized.

3.2.1. Optimization of separation

At first, the effect of varying the pH and composition of buffer was investigated. The following buffers were tried: sodium acetate, ammonium acetate, potassium acetate, zinc acetate, lithium citrate and magnesium acetate. The pH of the buffer was modified by acetic acid. Analysed premix sample consisted of four major peaks: salinomycin, 17-epi-20-deoxy salinomycin, 20-deoxysalinomycin and one unknown compound (labeled as S3). It was found that pH of the buffer and type of cation presented in buffer has a significant influence on the retention of all peaks of interest. Fig. 3 summarizes measured results.

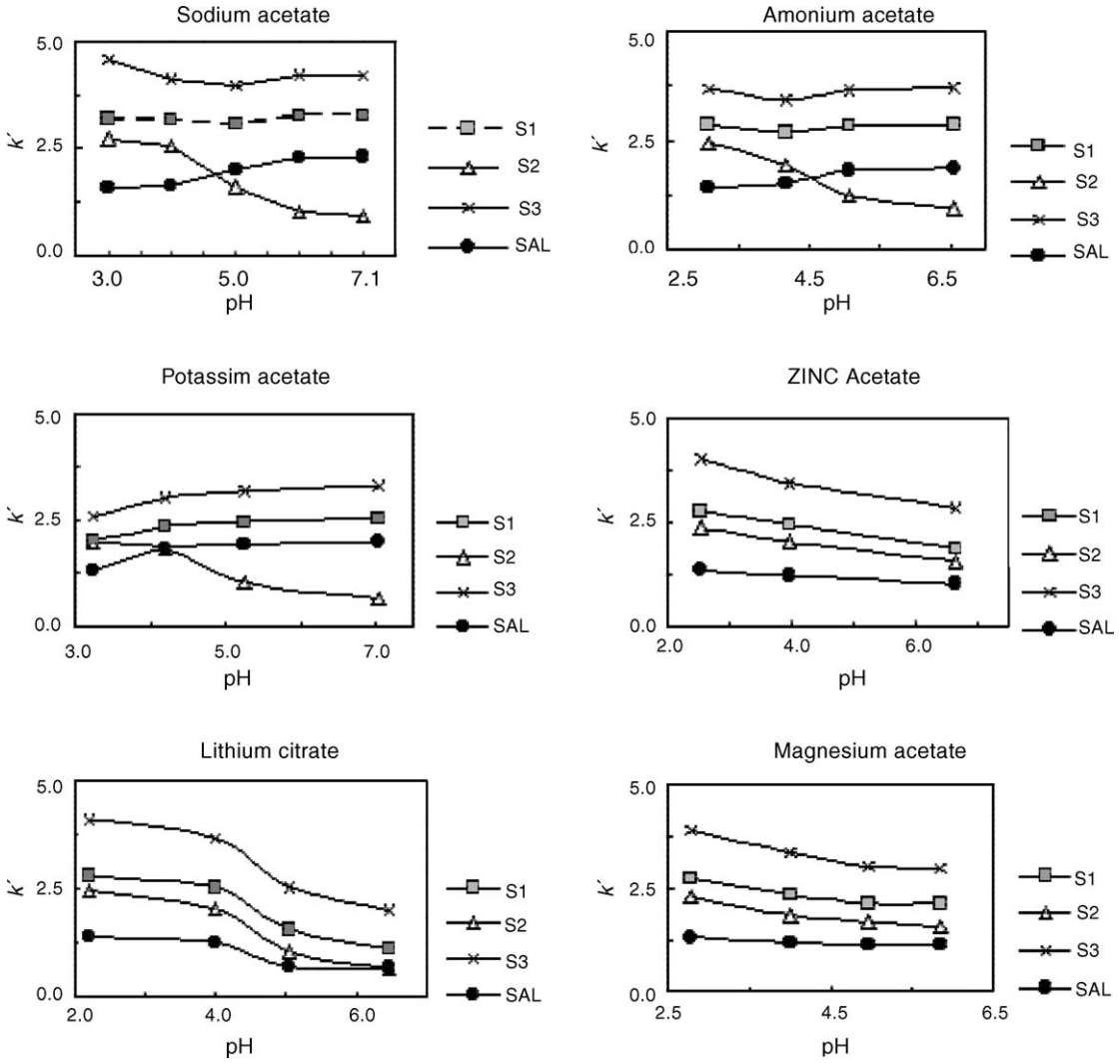

Fig. 3. Effect of composition and pH of buffers on retention factor of salinomycin (SAL), 20-deoxy salinomycin (S1), 17-epi-20-deoxy salinomycin (S2) and unknown compound (S3). LC conditions: column, Chromolith RP-18e (100 × 4.6 mm); mobile phase, methanol—0.05 M buffer (85:15, v/v); flow rate, 1.0 ml min^{-1}, post-column derivatization with vanillin reagent at 85 °C; flow rate, 0.5 ml min^{-1}, detection at 520 nm; analysed sample, methanol extract of 12% salinomycin premix (Biotika).

According to these observations, it is possible to divide the buffers in to the two groups. The first group consists of sodium acetate, ammonium acetate and potassium acetate. In their case with increasing pH, the retention factor (k') of salinomycin and 20-deoxy salinomycin slightly increases. On the other hand, rise of pH, caused rapid decrease of k' of 17-epi-20-deoxy salinomycin. The second group consists of lithium citrate, zinc acetate and magnesium acetate. In their case with increasing pH, the retention factor of all peaks decreased. These differences could be explained by dissimilar level of ionisation of carboxylic group in each buffer. It is know that ionophores can form dynamically reversible complexes with mono- and divalent cations. In these complexes carboxylic group forms head to tail hydrogen bond with terminal hydroxyl group. It could be assumed that in presence of sodium, potassium and even ammonium cation the carboxylic group of ionophores is blocked and thus has only little effect of their retention. Opposite situation is in second group of buffers when carboxylic group is not involved in complex formation and its ionisation at higher pH values tends to decrease of retention of all ionophores. As is shown the behaviour of 17-epi-20-deoxy salinomycin is in all buffers very similar and practically independent on composition and pH of mobile phase. This could be explained by permanent availability of the free carboxyl group, what is in good correlation with published data [26].

After taking all tried separation systems into consideration, the best resolution of all peaks of interest was achieved using zinc acetate buffer with pH 4.0.

3.2.2. *Optimization of detection*

The most characteristic factors that affect the post-column reaction are composition of reagent, reaction temperature,

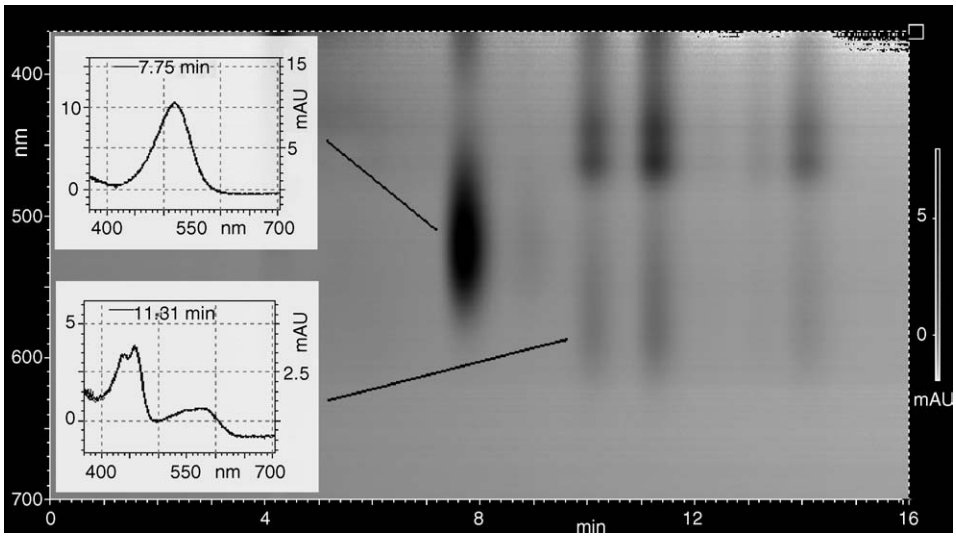

Fig. 4. Diode array chromatogram of methanol extract of 12% salinomycin premix (Biotika) after post-column reaction with VIS spectra of salinomycin (upper) and 17-epi-20-deoxy-salinomycin (lower) derivates, LC conditions: column, Chromolith RP-18e (100 × 4.6 mm); mobile phase, methanol—0.05 M zinc acetate buffer pH 4.0 (85:15, v/v); flow rate, 1.0 ml min^{-1}, post-column derivatization with vanillin reagent at 85 °C; flow rate, 0.5 ml min^{-1}.

dimensions and geometry of reaction coil and rates of reagent and mobile phase flows. In this study, we focused on the influence of reaction temperature and different flow rates of reagent and mobile phase on detection response, while the composition of reagent and dimensions of reaction coil were the same. The total flow rate was varied from 1.2 to 3.6 ml min^{-1} at the different ratio of mobile phase and reagent flow. In first experiment series, the flow of mobile phase was maintained at 1.0 ml min^{-1}, and the flow of reagent was varied from 0.2 to 1.0 ml min^{-1}. Reaction temperature was 80 °C and detector response was monitored using photo diode array detector (Shimadzu SPD-M10Avp) in the range from 370 to 700 nm. As is shown in Fig. 4 reaction product of vanillin with salinomycin has absorbance maxima at 520 nm, but reaction products of related compounds have maxima at 460 nm. Therefore, in further studies, simultaneous detection at 520 for salinomycin and 460 nm for related compounds was applied.

As expected, the flow rate of reagent had influence on detection response of monitored peaks. With the increase of the flow rate of reagent from 0.2 to 0.5 ml min^{-1}, the corrected area (A_c, A ml) of all peaks also increased. Further increase of reagent rate from 0.5 ml min^{-1} to 1.0 ml min^{-1} caused further increase of A_c of salinomycin (at 520 nm), but increase of A_c of all related compounds (at 460 nm) was not so steep. In the second series of tests, the flow of mobile phase was maintained at 2.0 ml min^{-1} and the flow of reagent was varied from 0.6 to 1.6 ml min^{-1}. In this case much lower response of all compounds was achieved. Results are summarized in Fig. 5.

Influence of the reaction temperature is shown in Fig. 6, when the increase of peak areas of all individual compounds with increasing reaction temperature was observed.

Although the best responses of all peaks were observed at highest temperature (90 °C), to avoid formation of bubbles, the temperature of reaction coil was maintained at 85 °C. In accordance with these results, the optimal ratio of mobile phase/reagent flow rate is 2:1. By increase of the total flow rate, the time of analysis decreases, but response area/height of all peaks decrease rapidly (Table 1).

Typical chromatograms of an ethanol extract of the fermentation broth collected at 60, 105 and 225 cultivation

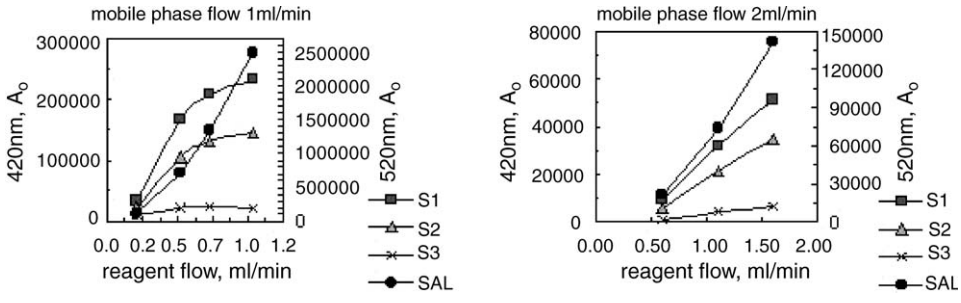

Fig. 5. Effect of reagent flow rate on response of derivates of salinomycin (SAL), 20-deoxy salinomycin (S1), 17-epi-20-deoxy salinomycin (S2) and unknown compound (S3) at mobile phase flow rate 1.0 ml min^{-1} and 2.0 ml min^{-1}. LC conditions are the same as in Fig. 4. Salinomycin derivate was monitored at 520 nm and derivate of related compounds at 460 nm.

Table 1
Effect of total flow rate on chromatographic parameters calculated for 20-deoxy salinomycin peak

Mobile phase flow (ml min^{-1})	Reagent flow (ml min^{-1})	Retention time (min)	Width	Height	Symmetry	Theoretical plates/m
1.0	0.5	11.6	1.23	8238	1.18	27519
2.0	1.0	5.9	0.69	4975	1.17	20580
3.0	1.5	4.0	0.46	2662	1.24	11378

LC conditions: column, Chromolith RP-18e (100 × 4.6 mm); mobile phase, methanol—0.05 M buffer (85:15, v/v), post-column derivatization with vanillin reagent at 85 °C, detection at 460 nm; analysed sample, methanol extract of 12% salinomycin premix (Biotika).

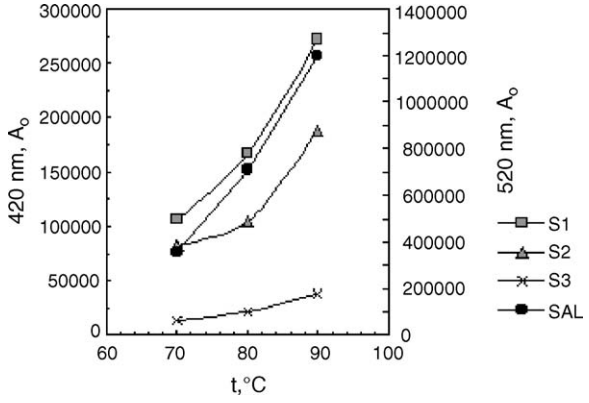

Fig. 6. Effect of reaction coil temperature on response of derivates of salinomycin (SAL), 20-deoxy salinomycin (S1), 17-epi-20-deoxy salinomycin (S2) and unknown compound (S3). LC conditions are the same as in Fig. 4. Salinomycin derivate was monitored at 520 nm and derivate of related compounds at 460 nm.

hours analysed under proposed chromatographic conditions are shown in Fig. 7.

Fig. 8 shows a comparison of chromatograms of 12% salinomycin premixes obtained from three different producers (Hoechst, Hoffman–La-Roche, Biotika), analysed under the proposed chromatographic conditions.

The linearity of the method was checked for salinomycin and 17-epi-20-deoxy salinomycin, analysing series of salinomycin standard concentrations in methanol: 0.10, 0.24, 0.49, 1.05, 1.26 mg ml^{-1} and series of 17-epi-20-deoxy salinomycin standard concentrations in methanol: 0.02, 0.05, 0.08, 0.18, and 0.28 mg ml^{-1}. Standard solutions were injected two times for each concentration level. Least-squares linear regression analysis of the data demonstrates good linearity over the examined range (Table 2).

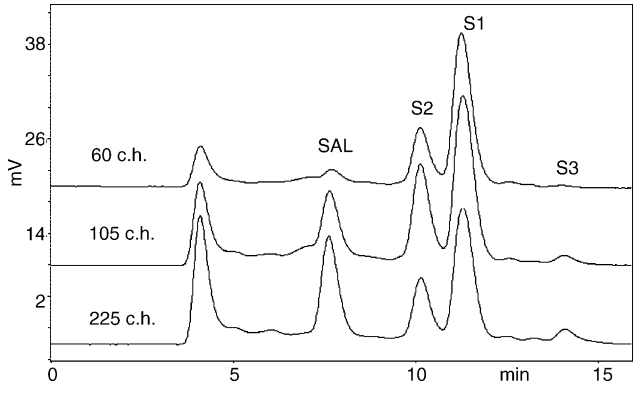

Fig. 7. Representative chromatograms of ethanol extracts of fermentation broth at different cultivations hours (60, 105 and 225 cultivation hours) at suggested LC conditions: column, Chromolith RP-18e (100 × 4.6 mm); mobile phase, methanol—0.05 M buffer (85:15, v/v); flow rate, 1.0 ml min^{-1}, post-column derivatization with vanillin reagent at 85 °C; flow rate, 0.5 ml min^{-1}, detection at 460 nm. SAL, salinomycin; S1, 20-deoxy salinomycin; S2, 17-epi-20-deoxy salinomycin and S3, unknown compound.

The intra-assay precision of the method was studied on the three premix samples with different concentrations of salinomycin (1, 12, 25%, w/w). The approximate salinomycin concentrations in the analysed solutions were 0.2, 0.9 and 1.2 mg.ml^{-1}. The intra-assay precision was then determined by replicate injections of six methanol extracts of each sample. Analysis of each sample was performed under the same conditions, utilizing the same equipment and chemicals, within a 24 h period. Results are shown in Table 3.

3.3. Solvent extraction

At first, the conditions of extraction were examined. With use of magnetic stirrer, when ethanol was used as extraction solvent, maximal extracted yield of salinomycin and co-products from premix sample were achieved after 45 min of mixing (at 300 RPM). Similar results were obtained in 10 min, when extraction was made in ultrasonic bath (35 kHz). Therefore, sonification was chosen as fastest and more convenient extraction technique.

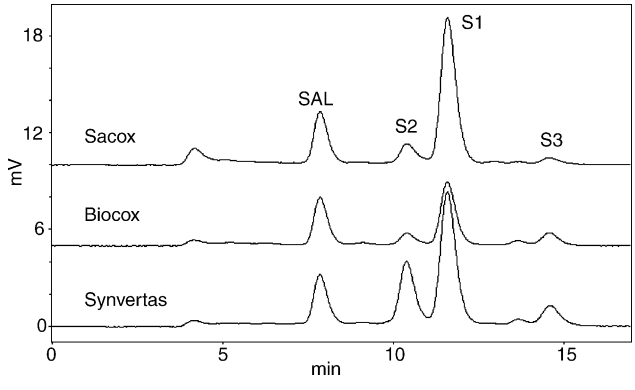

Fig. 8. Representative chromatograms of ethanol extracts of 12% salinomycin sodium premixes obtained from three different producers (Sacox (Hoechst, Germany), Biocox (Hoffman–La-Roche, USA) and Synvertas (Biotika, Slovakia)). LC conditions are the same as in Fig. 7. SAL, salinomycin; S1, 20-deoxy salinomycin; S2, 17-epi-20-deoxy salinomycin and S3, unknown compound.

Table 2
Linearity of the method

	Slope	Intercept (area)	Correlation coefficient (R^2)	Standard error of the slope	Standard error of the intercept
Salinomycin	27938	403	0.998	693	826
17-epi-20-Deoxy salinomycin	248213	−2932	0.995	7780	1734

Table 3
Intra-assay precision of the method

$n = 6$	Premix 1%			Premix 12%			Premix 25%		
	Mean ($g\,kg^{-1}$)[a]	S.D. ($g\,kg^{-1}$)[b]	R.S.D. (%)[c]	Mean ($g\,kg^{-1}$)[a]	S.D. ($g\,kg^{-1}$)[b]	R.S.D. (%)[c]	Mean ($g\,kg^{-1}$)[a]	S.D. ($g\,kg^{-1}$)[b]	R.S.D. (%)[c]
Salinomycin	11.0 ± 0.19	0.19	1.68	123.0 ± 1.56	1.49	1.21	255.5 ± 1.40	1.34	0.52
17-epi-20-Deoxy salinomycin	1.8 ± 0.04	0.04	2.10	18.0 ± 0.12	0.12	0.66	37.2 ± 0.25	0.24	0.64
20-Deoxy salinomycin [d]	2.6 ± 0.08	0.07	2.64	27.5 ± 0.23	0.22	0.79	55.3 ± 0.48	0.46	0.82
S3[d]	1.2 ± 0.03	0.03	2.84	14.5 ± 0.21	0.20	1.36	29.6 ± 0.25	0.23	0.79

[a] ± Confidence (at 95% level).
[b] Standard deviation.
[c] Relative standard deviation.
[d] Calculated as 17-epi-20-deoxy salinomycin.

Table 4
Comparison of extraction efficiencies of individual solvents

Solvent	Selectivity group [27]	Polarity index [27]	Salinomycin ($g\,kg^{-1}$)[a]	17-epi-20-deoxy salinomycin ($g\,kg^{-1}$)[a]	20-deoxy salinomycin ($g\,kg^{-1}$)[a,b]	S3 ($g\,kg^{-1}$)[a,b]	Sum ($g\,kg^{-1}$)
Diethyl Ether	I	2.8	114.2	15.2	27.7	6.0	165.9
2-Propanol	II	3.9	116.2	17.9	28.9	15.3	182.2
1-Butanol	II	3.9	110.6	18.3	26.5	14.6	173.9
Methanol	II	5.1	115.9	18.3	28.3	14.1	181.7
Ethanol	II	5.2	118.4	19.0	28.9	15.0	186.5
Tetrahydrofuran	III	4.0	119.4	16.6	27.6	12.9	180.5
Pyridine	III	5.3	116.0	19.6	29.8	14.6	185.3
N,*N*-Dimethylformamide	III	6.4	114.9	18.0	27.8	14.0	181.1
Dichloromethane	V	3.1	118.3	17.3	28.3	12.3	179.3
Acetone	VI	5.1	124.7	17.7	29.8	14.7	192.0
Acetonitrile	VI	5.8	111.4	9.9	26.5	13.2	166.8
Hexane	–	0.0	118.0	14.2	28.5	13.9	174.6

[a] Average concentrations of three replicate determinations.
[b] Calculated as 17-epi-20-deoxy salinomycin.

More solvents for extraction were chosen by their physical properties: selectivity, and polarity index. Reliability of the results was assured by three replicate extractions with each solvent. Results are summarized in Table 4 and Fig. 9.

Used solvents showed slightly different extraction selectivity for each compound. Acetone was found to be the best extractant for salinomycin and 20-deoxy salinomycin, pyridine for both 17-epi-20-deoxy salinomycin and 20-deoxy salinomycin and 2-propanol for S3. Similar extraction selectivity for all compounds showed ethanol, methanol and *N*,*N*-dimethylformamide. On the other hand 1-butanol and acetonitrile extracted least salinomycin and 20-deoxy salinomycin. Poor extraction efficiency showed acetonitrile for 17-epi-20-deoxy salinomycin and dietylether for S3. Recovery of all monitored compounds in examined solvents decreases in this order: acetone, ethanol, pyridine, 2-propanol, methanol, *N*,*N*-dimethylformamide, tetrahydrofuran, dichlormethane, hexane, 1-butanol, acetonitrile and diethyl ether.

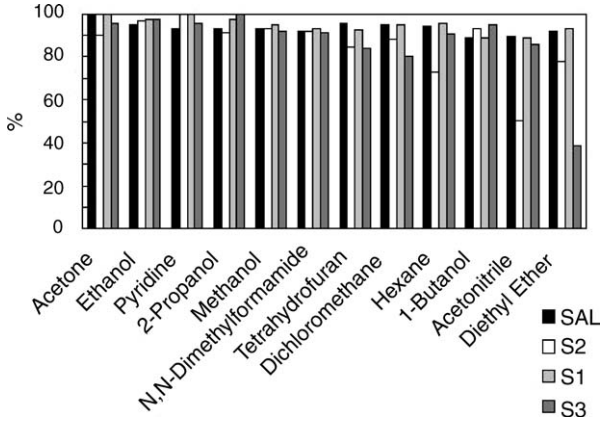

Fig. 9. Effect of different solvents on the efficiency of extraction of salinomycin and related compounds. Results are expressed as the percentage of the maximum amount extracted from premix sample. SAL, salinomycin; S1, 20-deoxy salinomycin; S2, 17-epi-20-deoxy salinomycin and S3, unknown compound.

4. Conclusion

The proposed HPLC method is suitable for monitoring of salinomycin and related compounds in fermentation media and premixes. Pre-column derivatizations of these compounds were shown as less suitable for determination, when compared with post-column derivatization with vanillin. Post-column derivatization with dual detection improved sensitivity of the detection and selectivity of the separation even without sample clean up. Rapid analysis was gained using monolithic type column. This method is shown to be precise and linear in the observed range. Acetone, ethanol and pyridine were found as best extraction solvent for salinomycin and related compounds. Method is currently used in laboratory scale for monitoring salinomycin productions.

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