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Review

# Survey of Planar Chromatography and HPLC Research in Croatia from 1980–1998

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The article presents the fundamental and applied research in planar and high-performance liquid chromatography in Croatia from 1980 to 1998. Fields and subfields of the investigations, as well as the places where they were done, are shown separately. Also, papers of individual investigators are elaborated. On the basis of the data presented, conclusions about the quantity and quality of the papers are drawn.

Key words: planar chromatography, HPLC, Croatia, review.

## INTRODUCTION

As seen from the title of this article, the scientific activity in planar chromatography (thin-layer chromatography, TLC, and paper chromatography, PC) and high-performance liquid chromatography, HPLC, in Croatia in the period of 1980–1998 is presented. The review of the papers published in this period was compiled by a computer-search of available databases: Science Citation Index (SCI) and Current Contents (CC). The citations are the result of the computer search based on a combination of key words relevant to planar and liquid chromatography (excluding gel-permeation and ion chromatography). Only papers stating that the first author's affiliation is

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with the institutions within the Republic of Croatia are covered in this survey. The papers published in journals that are not cited in SCI and papers published in symposium proceedings are not included, except the journal *Kemija u industriji*. Having in mind that Croatian chemists often publish their work in this journal, in order to get a more comprehensive survey of scientific production in Croatia, the papers published in *Kemija u industriji* are also included in this review.

### GENERAL CONSIDERATIONS

In the period from January 1, 1980 to August 31, 1998, a total of 105 publications from the field of TLC, PC and HPLC, covered by SCI and CC (including the journal *Kemija u industriji*), were published by the authors from the Republic of Croatia. The distribution of fields is shown schematically in Figure 1. Of these publications, 58% (61 papers) were related to the field of TLC, 39% to HPLC (41 papers) and only 3% (3 papers) to PC. The world productivity in these fields was very high during this period. A computer-based search of *Chemical Abstracts* found that more than 25000 papers reported research in planar chromatography and more than 70000 publications covered theory, techniques and applications of liquid chromatography (including gel-permeation, ion and supercritical fluid chromatography).

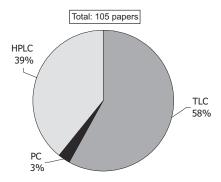


Figure 1. Classification of published papers according to the chromatographic technique employed.

The ratio of fundamental and applied research is shown in Figure 2. It can be observed that the applied research is predominant in both the planar chromatography and HPLC.

The classification of the published papers with respect to the area of analytical application is given in Figure 3. HPLC is mainly used in the analysis of environmental pollutants (32% of published papers) and as a helpful

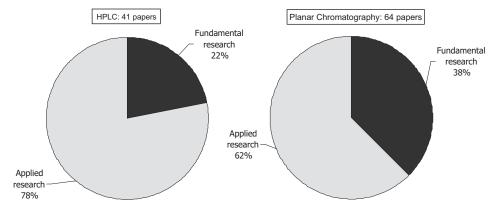


Figure 2. Classification of published papers according to the type of research performed.

tool in organic synthesis and analysis (31%). Planar chromatography is mainly used in inorganic chemistry (37%) of published papers) for analyses of heavy metals in different materials (alloys, ores, biological materials, *etc.*), in organic chemistry (22%) and in pharmaceutical chemistry (22%).

In addition, the analysis was done of the locations of investigations. This systemization was done according to the first author's affiliation in the case of cooperation of different institutions. As it is evident from Figure 4 HPLC is mostly performed in institutes (mainly Ruder Bošković Institute) where the instrumentation necessary for this kind of chromatography is situated, while half of all researches dealing with TLC is performed at the faculties, probably due to the fact that this technique is simple, not expensive and can

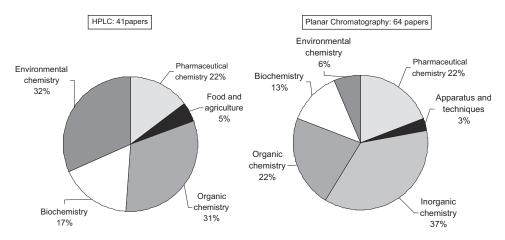


Figure 3. Classification of published papers according to the main area of research.

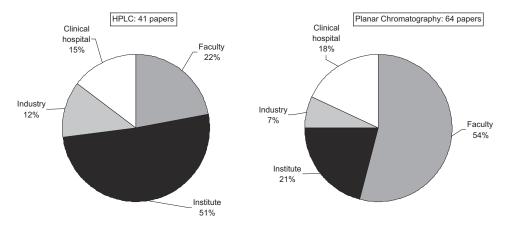


Figure 4. Classification of published papers according to the research institutions (based on the first author's affiliation).

be performed without sophisticated instrumentation. The scientific contribution of industrial researchers (PLIVA, INA *etc.*) is only 7% in the field of planar chromatography and 12% in HPLC. Among the institutions the highest production in the field of planar chromatography is at the Faculty of Chemical Engineering and Technology (24 papers), Ruđer Bošković Institute (9 papers) and Faculty of Pharmacy and Biochemistry (7 papers). In the field of HPLC, the highest production was at the Center of Marine Research of the Ruđer Bošković Institute (12 papers).

# **REVIEW OF ARTICLES**

#### Planar Chromatography (TLC and PC)

# Fundamental Studies of the Chromatographic System, Apparatus and Techniques

Several experimental studies of solute retention in TLC using modified layers were published: behavior of some phenolic acids, aldehydes<sup>1</sup> and benzene derivatives<sup>2</sup> on silica gel impregnated with iron(III), influence of progressive drying of iron(III) hydroxide oxide support on the behavior of some benzene derivatives,<sup>3</sup> retention of glucose and sorbitol on copper(II) impregnated silica gel plates,<sup>4</sup> retention mechanisms of substituted phenolic compounds on silica gel layers impregnated with Al<sup>III</sup> and Cu<sup>II</sup> ions,<sup>5</sup> separation of aminophenol isomers by metal-ion addition to the chromatographic layer,<sup>6</sup> formation of complexes between metal ions and some aromatic humic-like complexants in the chromatographic process simulating natural conditions,<sup>7</sup> retention of some metals on silica gel impregnated with phenolic acids,<sup>8</sup> and salicylic, syringic and *o*-phthalic acids,<sup>9</sup> mobility of Fe<sup>III</sup>-phenolic acid complexes,<sup>10</sup> phenol adsorption on active-carbon thin layers<sup>11</sup> and interaction between several essential and toxic metals and lignin impregnated paper.<sup>12</sup>

The following studies of mobile-phase optimization were reported: optimization of two-components systems by means of window diagrams,<sup>13</sup> application of numerical methods and information theory to the TLC analysis of flavonoids and phenolic acids in plant extracts<sup>14,15</sup> and chamomile essential oil,<sup>16</sup> application of three mathematical techniques (calculation of the information content, the discriminating power and cluster analysis) for the evaluation of solvent combinations in TLC.<sup>17,18</sup>

Although many researchers use TLC on qualitative and semiquantitative basis, modern computer-controlled densitometers allow precise and accurate quantitative analysis. Validation of the results obtained has become increasingly important. Publications on the validation of quantitative TLC were: validation of quantitative chromatographic analysis on laboratory prepared thin-layers using color analyzer, image analyzer and slit-scanning densitometer,<sup>19</sup> validation of quantitative TLC analysis by visible region densitometry of dye samples using color analyzer.<sup>20</sup>

### Applications

Pharmaceuticals, Drugs and Alkaloids. – Planar chromatography was used for the isolation of glucosides from mistletoe leaves (Viscum album L.),<sup>21</sup> for identification of aglycones of beta-D-glucosides from the leaves of Dalmatian sage (Salvia officinalis),<sup>22</sup> for isolation of flavonoid glycosides from the methanolic extract of the leaves of Christ's Thorn,<sup>23</sup> some derivatives of 4,7-dihydroxycoumarins were separated by TLC.<sup>24</sup> Thin layer spectrodensitometry was used for quantitative determination of carboprost in sterile solutions,<sup>25</sup> for determination of betamethasone dipropionate in semi-solid pharmaceutical preparations,<sup>26</sup> HPTLC analysis of glibenclamide in tablets was compared with HPLC,<sup>27</sup> while butylated hydroxytoluene (BTH) was determined in gum base by slit-scanning densitometry.<sup>28</sup>

*Biochemical Applications.* – High-performance radial thin layer chromatography was used for semi-quantitative determination of drugs in urine.<sup>29,30</sup> The following compounds were quantified by TLC: tricrynafen (tienilic acid) in dog plasma,<sup>31</sup> urinary testosterone, epitestosterone and androstenedione,<sup>32</sup> lipids in plasma filtrates obtained in therapeutic filter plasmopheresis,<sup>33</sup> muramic and diaminopimelic acids in acid hydrolysates of fermentation broth,<sup>34</sup> unconjugated cholic, chenodeoxycholic, deoxycholic and lithocholic acids,<sup>35</sup> gangliosides in human cerebrospinal fluid<sup>36</sup> and C-series polysialogangliosides in human cerebella.<sup>37</sup>

Various Organic Compounds. – Different organic compounds were separated and quantified on TLC plates (on silica gel layers unless otherwise noted): carbohydrates in the first and final stages of isolation of enzyme peroxidase from Horseradish root,<sup>38</sup> air-borne carbofuran and quinalphos,<sup>39</sup> metrifonate and DDVP in rats,<sup>40</sup> aquatic humic acids,<sup>41</sup> different types of surfactants of all four groups (anionics, cationics and amphoterics),<sup>42</sup> dye (C.I. Reactive Red 120) and its hydrolyzed by-products,<sup>43</sup> combination of agrochemicals (atrazine, propham, chlorpropham, diflubenzuron,  $\alpha$ -cypermethrin and tetramethrin) in spiked soil samples.<sup>44</sup>

*Inorganics and Metal-organics.* – TLC-densitometry was used for quantitative analysis of alloys and metal containing materials: zirconium was determined in bauxite and aluminum alloys,<sup>45</sup> magnesium <sup>46</sup> and zinc were determined in aluminium alloys along with anodic sampling, <sup>47</sup> silver in aluminium,<sup>48</sup> aluminum and magnesium were visualized by a flow of 2-(salicylideneamino)phenol or 2-hydroxybenzaldehyde and (2-hydroxybenzyl)hydrazone and quantified using frontal detection,<sup>49</sup> magnesium in Al-alloys was separated on chromatographic plates coated with Amberlite IRP-69 (strongacid cation exchanger),<sup>50</sup> platinum and rhodium were quantitatively determined in Pt-Rh catalysts,<sup>51</sup> and dental base alloys using anodic sampling.<sup>52</sup>

The following studies on the chromatographic behavior of different metals were reported: study of iron(III) and gold(III) separation by paper chromatography,<sup>53,54</sup> separation and identification of antimony(III), antimony(V), arsenic(III) and arsenic(V) by TLC.<sup>55</sup> Separation and identification of cations were investigated in six analytical groups,<sup>56,57</sup> while Au<sup>3+</sup>, V<sup>5+</sup>, Tl<sup>+</sup>, Tl<sup>3+</sup>, Ce<sup>4+</sup>, Th<sup>4+</sup>, UO<sub>2</sub><sup>2+</sup>, Pt<sup>4+</sup>, Se<sup>4+</sup> and Te<sup>4+</sup> were separated on cellulose layer.<sup>58</sup> The reactions of inorganic ions and numerous organic reagents were investigated on microcrystalline cellulose and on silica gel.<sup>59,60</sup>

The use of TLC and densitometry was reported in the quantitative determination of heavy metals in textile materials: traces of copper, iron and manganese in cotton material using wet digestion<sup>61, 62,63</sup> and in wool material after dry combustion.<sup>64</sup>

#### Liquid Chromatography (HPLC)

## Fundamental Studies of Retention Behavior

Correlation of structure and retention behavior in reverse phase HPLC was studied in two papers: for methionine enkephalin related glycoconjugates<sup>65</sup> and for leucine enkephalin related glycoconjugates.<sup>66</sup>

Topological indexes were applied in the following studies: calculation of chromatographic properties for polycyclic aromatic hydrocarbons using the Wiener number (W) as parameter,<sup>67</sup> prediction of chromatographic behavior of alkanes,<sup>68</sup> calculation of retention times of anthocyanins with orthogona-lized topological indexes.<sup>69,70</sup>

# Applications

*Environmental Chemistry.* – The following studies employing HPLC in environmental chemistry were published: linear alkylbenzensulphonates were determined in the Krka River estaury,<sup>71</sup> nonylphenoxycarboxylic acids in sewage effluents,<sup>72</sup> alkylphenol polyethoxylate surfactants in the aquatic environment (rivers and during sewage treatment),<sup>73,74</sup> toxic metabolites from nonionic surfactants in the Krka river estaury,<sup>75</sup> linear alkylbenzensulphonates (LAS) and their persistent metabolites in the highly stratified Krka river estaury,<sup>76,77</sup> in estuarine mixed bacterial cultures,<sup>78</sup> and during infiltration of river water to groundwater,<sup>79</sup> chlorophyll and carotenoid pigments in stratified Krka estuary and in Northern Adriatic,<sup>80,81,82</sup> pesticide atrazine in drinking water in pig-breeding farm surroundings.<sup>83</sup>

*Pharmaceuticals, Drugs and Alkaloids.* – Several authors used HPLC for the determination of pharmaceuticals and similar compounds: reversed phase HPLC was used for separation of delta-2 and delta-3 isomers of 7-adca and cephalexin monohydrate,<sup>84</sup> HPLC determination of cephalexin was compared with microbiological methods,<sup>85</sup> semisynthetic macrolide antibiotic azithromycin was analyzed,<sup>86</sup> labetalol in biological materials was detected using HPLC with electrochemical detection,<sup>87</sup> atenolol using HPLC with fluorescence detection<sup>88</sup> and kinetics of hydrolysis of ketoprofen was studied by HPLC using diclofenac as the internal standard.<sup>89</sup>

Biochemical Applications. – HPLC was applied in the following studies of biochemically important compounds: determination of serum oxprenolol,<sup>90</sup> plasma 17- $\alpha$ -hydroxy-progesterone,<sup>91</sup> serum diclofenac<sup>92</sup> and imipramine,<sup>93</sup> serotonin in peripheral rat tissues<sup>94</sup> and ochratoxin A in serum.<sup>95</sup> Coupled chiral and achiral HPLC was used for the determination of plasma levels of R-(+)-amlodipine and S-(–)-amlodipine after single enantiomer administration.<sup>96</sup>

*Various Organic Compounds.* – HPLC was used for the determination of supplemental methionine<sup>97</sup> and butylated hydroxytoluene<sup>98</sup> in poultry premix. The separation of enantiomers by liquid chromatography was studied on triacetylcellulose.<sup>99</sup> The principles and applications of separation of enantiomers on analytical and preparative scale were rewieved.<sup>100,101</sup> The preparative separation or enrichment of chiral 2H-chromenes was accomplished by enantioselective HPLC on triacetyl- or tribenzoylcellulose.<sup>102,103</sup> The flavan fingerprints were determined in plant extracts<sup>104</sup> and selected alkaloids (nicotine and nornicotine) and sugars in tobacco extracts.<sup>105</sup>

#### CONCLUSIONS

Chromatography is a traditionally well represented subfield in the research activities of analytical chemists in Croatia. Since the beginning of 1960s several Croatian researchers have been internationally recognized as experts in this field. In the field of planar chromatography, we have to mention the outstanding contribution of dr. Srećko Turina, one of the pioneers of modern TLC.

From close examination of the data presented in this review, several conclusions can be drawn: the quality of published papers is satisfactory, they are mainly published in high ranked journals (*Journal of Chromatography, Chromatographia, Journal of Liquid Chromatography)*. However, the quantity is not satisfactory (the average is 5.83 papers per year). Especially low productivity was recorded in the field of HPLC, which is understandable taking into account that majority of our scientific institutions, particularly faculties, are poorly equipped. The scientific contribution of industrial institutes is very low (only 10 papers in almost 18 years). This may imply that researchers in industrial institutes are preoccupied with other duties and not stimulated to publish their work.

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# SAŽETAK

#### Pregled istraživanja na području plošne kromatografije i HPLC u Hrvatskoj od 1980. do 1998.

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Dan je pregled fundamentalnih i primijenjenih istraživanja na polju plošne kromatografije i tekućinske kromatografije visoke djelotvornosti (HPLC) u Hrvatskoj od 1980. do 1998. Posebno su prikazana pojedina područja istraživanja, uz navođenje radova pojedinih autora. Na toj su osnovi izvedeni zaključci o kvaliteti i kvantiteti objavljenih radova.