## Magic Square. the Usage of Double Bonds Photoisomerization for Indentification of Organic Compounds by Thin-Layer Chromatography

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# MAGIC SQUARE. THE USAGE OF DOUBLE BONDS PHOTOISOMERIZATION FOR INDENTIFICATION OF ORGANIC COMPOUNDS BY THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

The technique of chromatography is presented which enables to combine chromatographic separation and identification of organic compounds, containing double-bonds. The method is based on different chromatographic mobility of Z- and E-isomers. Isomerization is carried out by direct surface UV-light irradiation on sorbent. This method can be successfully applied to identification of aromatic azo- and azoxycompounds, stilbene derivatives and unsaturated acids.

INTRODUCTION

Identification in TLC is carried out when $R_f$ values of an unknown compound and standard are equal. Visual colour comparison of spots is one more way of identification for coloured compounds. The visualised reagents treatment of chromatograms and the usage of fluorescent indicators should be attributed to the same aim ( 1,2 ).

The double-bonds-containing substances are able to reversible
Z - E isomerisation in solutions. This property may be used for
plate chromatography identification. TLC of organic compounds,
existing in isomeric or tautomeric equilibrium, has several speci-
fic features. The main characteristics of tautomerides, seporated
in thin layer, were described by Marcinkiewicz ( 3 ). The decisive
feature influencing chromatography picture is the rate of tauto-
merising. Slow convertion of absorbed compounds leads to separa-
tion into individual spots. The strips in the direction of elution
are formed on the plates when development and isomerization are
comparable in time. When there is a very high rate of tautomer
interconversion, both isomers move as one spot and no separation
can be observed.

One can naturally presume the analogous chromatography beha-
viour in case of geometric isomers. However  the activation ener-
gy of Z - E isomerization is higher, as a rule, than of tautome-
rization. The later makes it possible to separate chromatographi-
cally geometrical isomers.

The classical method of energeticaly non-profitable Z-isomers
preparation is based on UV-light irradiation of E-isomers com-
pounds with a wave length of the electronic $\pi - \pi^*$ or n $-\pi^*$ transi-
tion. The relative isomer  content in mixture tends to a definite
value in time - photostationary state. Isomerization also takes
place when light affects on the absorbed substances ( 4 ). The
above mentioned facts enable us to suggest the identification
method of organic compounds, isomerized slower, than eluted. This
method is based on the combination of UV-irradiation and two-di-
mentional chromatography.


## EXPERIMENTAL

The simpliest organics, represent classes of ethylenedicar-
bonic and phenylethylenecarbonic acids, 1,2-diphenylethylens,

aromatic azo-compounds and azoxycompounds were studied. Dibutyl-
maleinat, E-stilbene, E-azobenzene and E-azoxybenzene were used
for the experiments.

The corresponding Z-isomers were prepared by photochemical
reaction ( 5,6 ). Besides, methyl esters of cinnamic and ferulic
acids produced by diazomethane methylation in ether in the presence
of methanol were studied. The compounds of commercial grade purity
were used in the experiments.

Merck N5721 plates with fixed silica gel layer were used for
chromatography. The elution was carried in chambers with satura-
tion, by organic solvent mixtures in standard conditions. There
were used solvents freshdistilled in glass. Using disposable,
open - end cappilaries ( Carl Zeiss, Oberkochen, 7082 ), 5 ml of
sample solution were applied to the TLC plate. The plates were
irradiated by mercuryquartz lamp. The plates were scanned in UV
and visible wave regions at spectrodensitometer KM-3. Electron
spectrum  of diffuse reflection were registered by the same device.

## CHROMATOGRAPHICAL METHODS

The spots were placed in the left corner of the plate at the
distance of 2 cm from the edge, exposing during 5 min in the air
and 15 min under the source of UV-light at the distance of 20 cm.
Then  the plates were cooled in darkness during 10 min and developed
in the light - protected chamber. The eluents and $R_f$ values of the
studied compounds after the first chromatography are listed in
table. Afterwards the plate was once more irradiated, observing the
mentioned conditions, and developed in the perpendicular direction.

## RESULTS AND DISCUSSION

The first chromatography. As it was discussed by Klemm and
Snyder ( 7,8 ), the interaction between adsorbed organic substrate

and oxide sorbents surface is realised by the donor - acceptor
bonds. The observed differences of $R_f$ values Z- and E-isomers ref-
lect the effect of geometrical structure on the electron distribu-
tion in their molecules as well as the change of their spatial
orientation in relation to sorbent surface.

According to the experimental data (7,8) Z-isomers are eluted
later, than E-, in case of ethylenedicarbonic acids. Probably, it
is explained by the affect of carboxyl group space disposition on
the acid strength ( field effect ), and stronger interaction of
the carboxyls with sorbent, forming chelate structure in Z-form.
Such interaction with sorbent surface leads to the observed $R_f$
values of maleic and fumaric acid dibutyl esters. (See table).

It is energetically more advantageous for E-azobenzene to have
molecular plane order as to sorbents surface, that provides the
effective interaction of the conjugate $\pi$-electron system with sur-
face groups. The plane structure is disturbed in Z-form because of
the phenyl substituents steric repulsion. So orthogonal molecular
orientation turns out to be advantageous. $R_f$ values of azocompounds
indicate greater interaction of n-pairs nitrogen atoms than $\pi$-elec-
trons with sorbent. The same phenomenon is observed in case of azo-
xybenzene isomers chromatography. Evidently, non-divided electron
pairs of oxygen and nitrogen provide interaction with a surface.

If there are no n-electrons containing atoms in molecule (styl-
benes), or only one n-donor centre (cinnamic or ferulic ether), the
conjugate $\pi$-system is determinative in surface interaction. It
is a well known fact, that molecular plane of Z-configuration is
diminished by steric repulsion of substituents. So effective over-
lapping of atomic p-orbitals in Z-form is less, than in E-isomer.
For stylbene, cinnamic and ferulic acid esters, E- has less $R_f$
value than Z-isomer. Geometrical isomers of azobenzene, azoxybenzene,
dibuthyl esters of ethylenedicarbonic acides are characterised
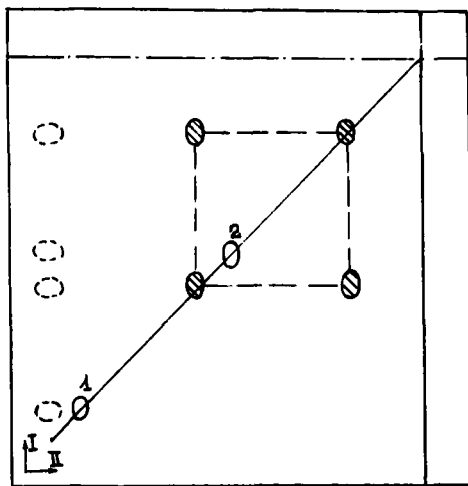by reverse sequence of elution.

FIGURE. Magic square of azobenzene (hexane : acetone 95:5,
      Merck, $SiO_2$).

   ⊘ - isomerised spots of azobenzene;

   O - non-isomerised spots of p-aminoazobenzene-1 and Sudan-2;

     - magic square.

      The order of development is shown by arrows.

   The second chromatography. After the second irradiation and
chromatographic procedure, which is done in the perpendicular di-
rection each spot subdivided in two, with relatively equal $R_f$
values.*

   By means of chromatography each spot was refered to Z- or E-form
according to the $R_f$ value of speciemen thus, each substance turned
to isomer mixture by irradiation. The typical chromatogram is shown
on the picture. There are specifically situated spots on the plate-
in the corners of the square. Line drown through the start and

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   *The subdivision of azoxybenzene in three spots was observed.
Yellow spot is resulting product of Vallach rearrangement and is
identified as 2-oxyazobenzene.

TABLE

Chromatographic Behaviour of Isomerising Compounds,

Containing Double Bonds.

| N | Substance | $R_f$ | | Plate, sorbent | Eluent |
|---|-----------|-------|---|---------------|--------|
| | | Lower spot | Upper spot | | |
| 1. | Stilbene | 0,21 (E) | 0,28 (Z) | Merck, $SiO_2$ | Hexane |
| | | 0,34 (E) | 0,53 (Z) | Merck, $Al_2O_3$ | _"_ |
| 2. | Difluorostil-bene | 0,3 (Z) | 0,41 (E) | Merck, $SiO_2$ | Hexane |
| | | 0,58 (Z) | 0,75 (E) | Merck, $Al_2O_3$ | _"_ |
| 3. | Azobenzene | 0,42 (Z) | 0,80 (E) | Merck, $Al_2O_3$ | Hexane: Acetone 95:5 |
| | | 0,40 (Z) | 0,79 (E) | Merck, $SiO_2$ | _"_ |
| 4. | 4-Nitroazoben-zene | 0,21 (Z) | 0,58 (E) | Merck, $SiO_2$ | Hexane: Acetone 19:1 |
| 5. | Azoxybenzene | 0,18 (Z) | 0,67 (E) | Merck, $SiO_2$ | Hexane: Acetone 10:1 |
| 6. | Dibuthyl Ester of Ethylenedi-carbonic Acid | 0,51 (Z) | 0,85 (E) | Merck, $SiO_2$ | Hexane: Acetone 10:1 |
| 7. | Methyl Ester of Cinnamic Acid | 0,65 (E) | 0,75 (Z) | Silufol, $SiO_2$ | Hexane: Acetone 3:1 |
| 8. | Methyl Ester of Metoxyferu-lic Acid | 0,77 (E) | 0,83 (Z) | Silufol, $SiO_2$ | Hexane: Acetone 7:3 |

cross point of solvent boundaries intersects the square by diagonal.
All spots belonging to non-isomerised compounds are situated on
this straight line too. Formation of the square proves the presence of
isomerised compound in mixture under UV-irradiation i.e. contain-
ing unsaturated bonds in molecule. We called symbolically such
identification method as a "magic square".

## CONCLUSION

Reversable photoisomerisation phenomenon of Z, E-isomers of
stylbene, azobenzene, azoxybenzene, dibuthyl esters of ethylenedi-
carbonic acids, methyl esters of cinnamic and ferulic acids ab-
sorbed on the surface of chromatographic sorbents is proved. This
ability is used as a specific reaction. UV-irradiation turns off
the spots of individual isomers into isomeric Z,E-mixture, which
separates by chromatography.

Besides $R_f$ value identification, the comparison of electrone
diffuse reflection spectrum  of photoreaction products and indi-
vidual isomers in absorbed state was performed.

"Magic square" identification method will be useful in case
of complex samples, containing compounds with close $R_f$ values
and colouring. Moreover, this method can be used without standard
for isomerised compounds in complex mixture.

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