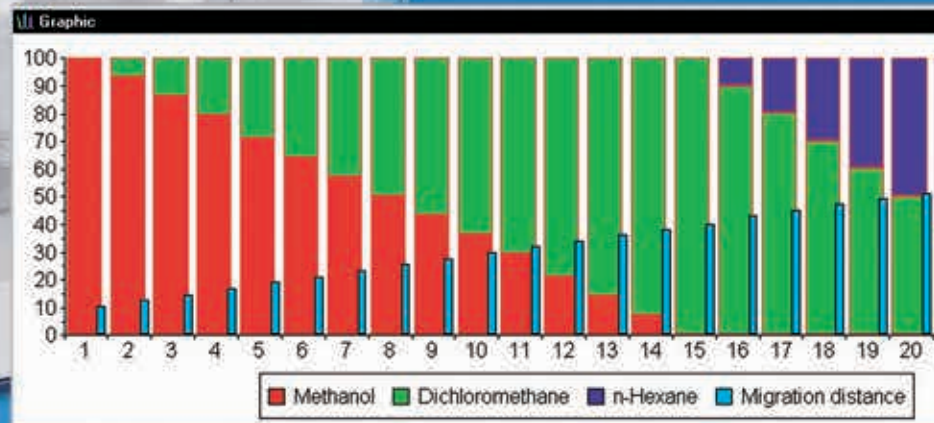
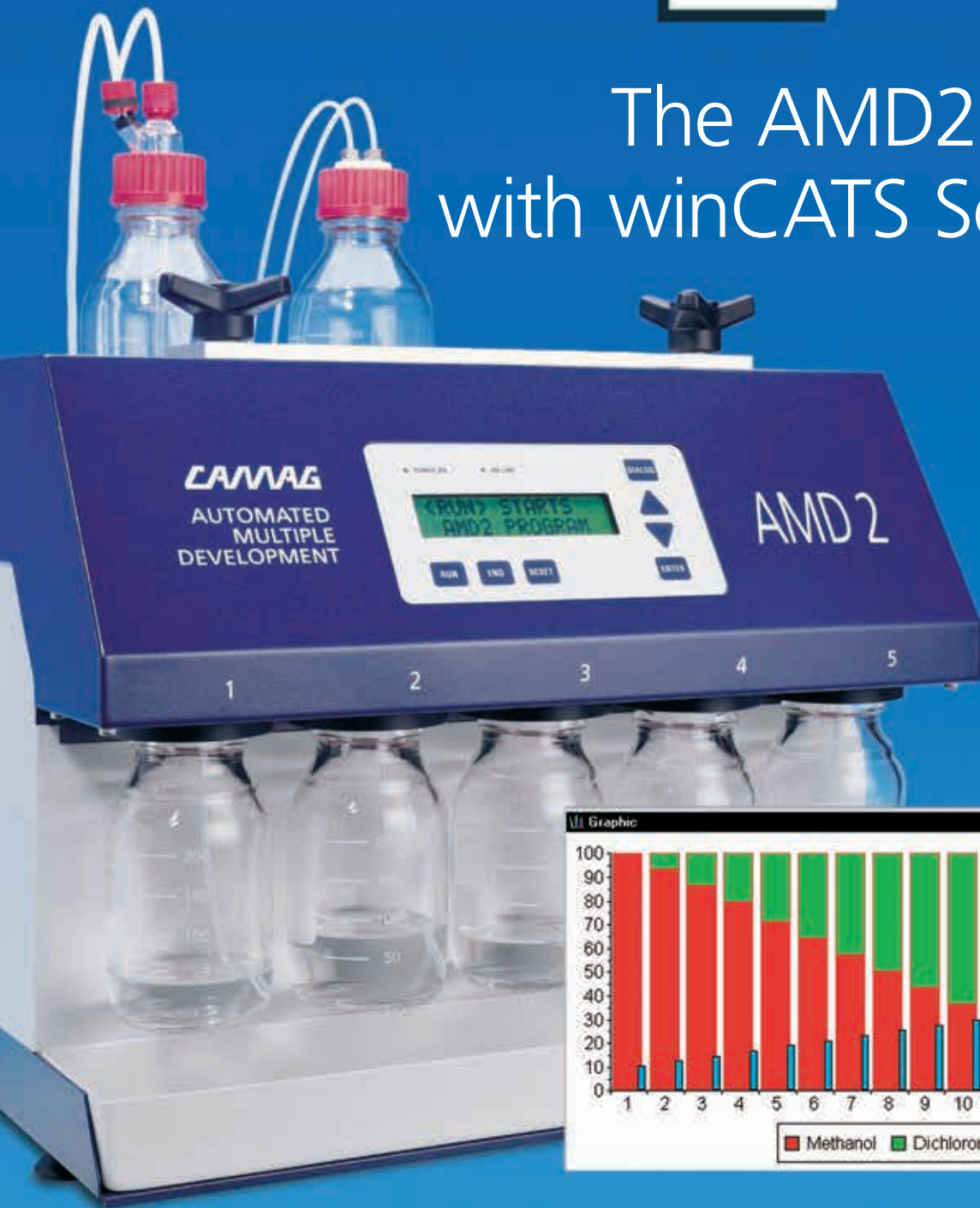




The AMD2 System with winCATS Software



Gradient Elution
in Planar Chromatography
Automated Multiple Development of
Thin-layer Chromatograms

What are the advantages of Automated Multiple Development

AMD = Automated Multiple Development

The CAMAG AMD procedure allows thin-layer chromatography to be utilized for tasks that could not be performed by TLC in the past.

AMD offers:

- gradient elution
- separation power increased by a factor of 3 compared with regular HPTLC
- improved precision and reliability
- after-hour development.

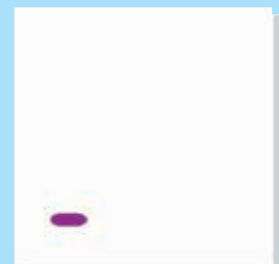
Only the AMD procedure can be successfully employed for reproducible gradient elution with silica gel as the stationary phase. In column liquid chromatography, gradient elution is common on reversed phases only because a silica gel column would call for a time consuming reconditioning or be irreversibly degraded, which is not acceptable in a technique depending on multiple use of the stationary phase. In planar chromatography this is not relevant.

The principle of the CAMAG AMD procedure

- The HPTLC plate is developed repeatedly in the same direction
- Each successive run extends over a longer solvent migration distance than the one before
- Between runs, the solvent is completely removed from the developing chamber and the layer is dried under vacuum
- Each successive run uses a solvent of lower elution strength than that of the one used before. In this way, a stepwise elution gradient is formed
- The combination of focusing effect and gradient elution results in extremely narrow bands. Their typical peak width is about 1 mm. This means that, with the available separation distance of 80 mm, up to 40 components can be completely resolved, i.e. with base line separation.

AMD is focusing chromatography

a) *Substance zone between two runs*



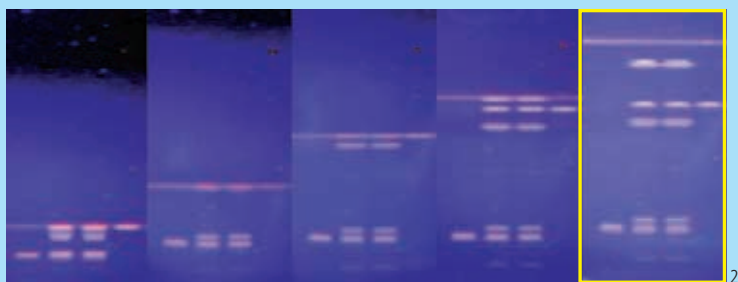
b) *The lower part of the substance zone is already taken up by the upward movement of the mobile phase and starts migrating in the direction of chromatography while the upper portion is still at rest*



c) *The fraction is focused to a narrow band.*



Development of Thin-Layer Chromatograms?

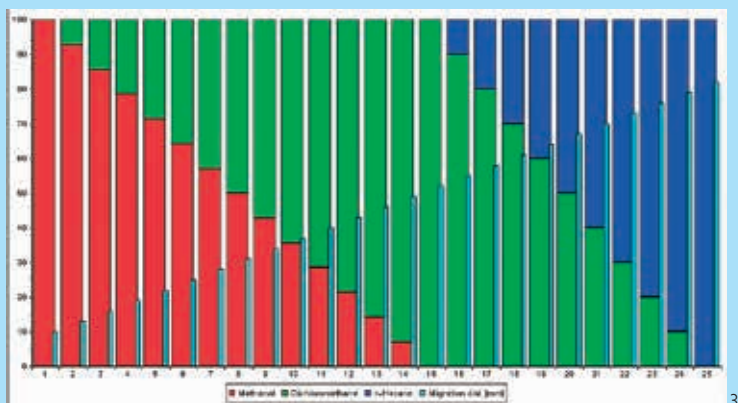


This series – shots taken after various steps in the gradient – illustrates the effect of the AMD separation technique.

Here lipids were chromatographed with the universal gradient shown in the diagram. With the first plate, the developing process was interrupted after 5 steps. The chromatogram was derivatized and documented. A second plate was developed over 10 steps, a third over 15, a fourth over 20, up to 25 steps for the fifth.

Initially, as can clearly be seen, all fractions migrate with the front or closely behind it. With decreasing strength of the solvent, one fraction after the other detaches from the front zone and remains almost stationary in the following runs.

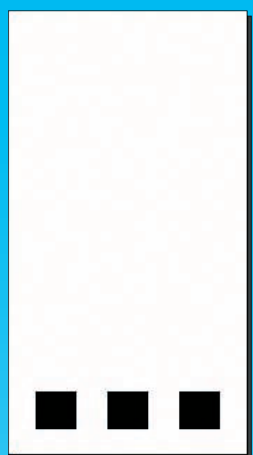
High separation efficiency is achieved in this way. Separation is reproducible, and the distance that a given fraction migrates before stopping is largely independent of the sample matrix.



Universal gradient: Unlike a gradient in column liquid chromatography, the AMD gradient starts with the strongest, in this case the most polar solvent and is varied toward decreasing elution strength. The most polar elution solvent is run over the shortest developing distance, the most non-polar over the longest.

“Universal gradient” is the term for a normal phase AMD gradient that comprises the whole polarity range of solvents, i.e. starts with a very polar solvent, continues with solvents of medium polarity and ends with non-polar solvents, so that substances from virtually all classes of compounds are chromatographed. A shallow gradient is suitable for the separation of closely related substances.

If extremely large sample volumes (50–100 μL) have to be applied, this is best accomplished by spraying-on rectangles with the CAMAG Automatic TLC Sampler 4 (ATS 4). This prevents the layer from becoming washed away by excessive solvent per unit area. Another advantage of this technique is the distribution of matrix across a larger area.



Samples applied as rectangles with (e.g. lines within a rectangle)



The same samples focused to narrow bands, which is accomplished in AMD chromatography during the initial developing steps using strongly eluting mobile phases.

The CAMAG AMD2 System

The AMD2-System consists of the developing module and the AMD2-EquiLink for winCATS. For operation also required is the winCATS software and a vacuum pump, preferably an oil sealed rotary vane pump.

Function

An AMD developing step proceeds as follows:

- The solvent made up in the gradient mixer is released into the developing chamber containing the HPTLC plate; migration of the solvent in the layer starts immediately, uniformly over the total width
- The position of the solvent front is monitored by a CCD element. Upon reaching the height programmed for this step, chromatography is interrupted
- This is achieved by withdrawing the solvent from the chamber into the waste bottle by partial vacuum; then full vacuum is applied for drying the HPTLC plate
- Before the next developing cycle is started, vacuum is released and if required the chromatogram layer is preconditioned with a defined gas phase
- Parallel to this cycle, the programmed solvent for the next step is made up in the gradient mixer.

The complete program is fully automatic; no supervision is required. The pump is set to run after completion of the AMD program until solvent from the pump oil system is evaporated.

Developing module

The actual developing chamber is situated in the front portion of the housing. Five solvent reservoir bottles are arranged in front. A gas wash bottle and a solvent waste bottle are integrated in the hood. All valves, the gradient mixer, connecting elements and the electronic processor are accommodated inside the housing.



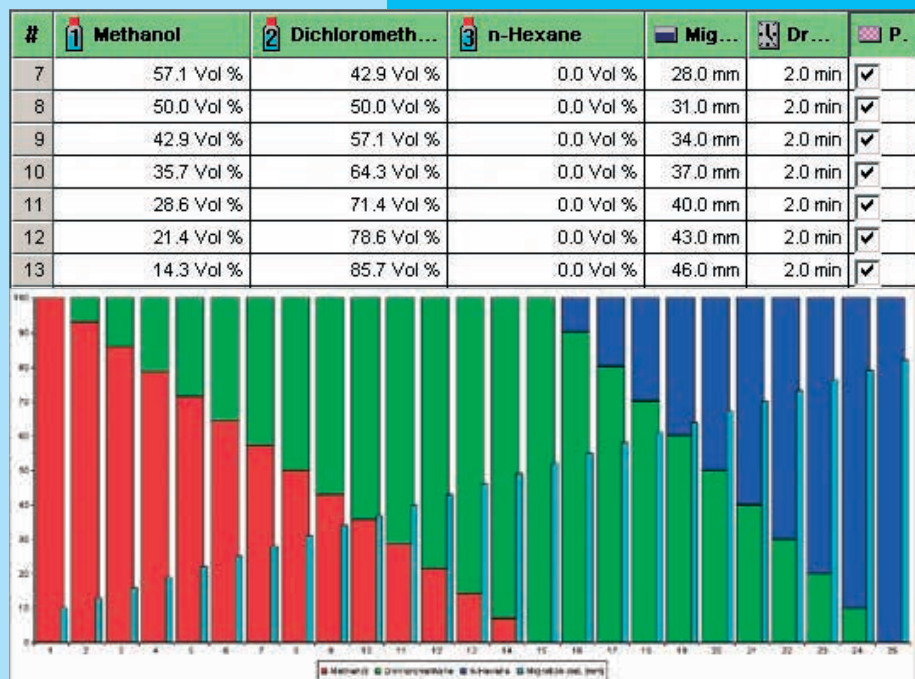
Programming/Control

A complete development procedure can easily be entered into a spreadsheet on the PC and the result is depicted graphically for review. When the gradient program begins, all parameters are transmitted to the developing module, which continuously updates the gradient status to the computer.

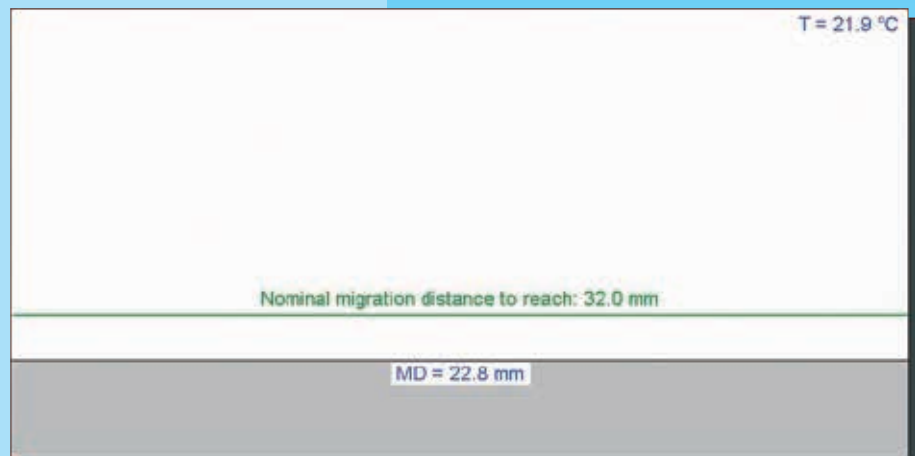
The developing module (Fig. 6) can also operate in "stand alone" mode, i.e. the most recently used developing program can be repeated as often as desired.

Entering the gradient

The gradient is built from up to 5 solvent bottles by entries into this table. Also desired developing distances, drying times and preconditioning are entered. The gradient, including developing distance of the individual runs, is graphically displayed for verification. (Fig. 7).



The window "Development" displays the current status of the program ▼



How to optimize an AMD gradient

In the past, a wealth of chromatographic experience was required to find a suitable solvent system for a given separation problem. With the AMD technique, optimization of the mobile phase becomes routine. This is shown using the analysis of sulfonamides in animal feed as example.

For therapeutic reasons sulfonamides and antibiotics are administered to animals in the form of so-called feed supplements. The assay of active ingredients in animal feed is regulated by law. Due to the complex matrix of the samples to be analyzed, a powerful and reliable separation technique must be employed.

Apart from its increased separation power, AMD has two distinctive features useful for this purpose:

1. When the densitogram curve of a chromatogram is superimposed with the in-scale diagram of the gradient, the region of the gradient causing the separation of given fractions can be identified.
2. The migration distances of the individual components are independent of the sample matrix, which is not the case in normal, one-step TLC development.

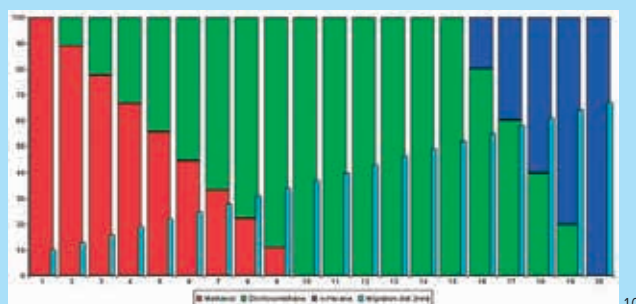
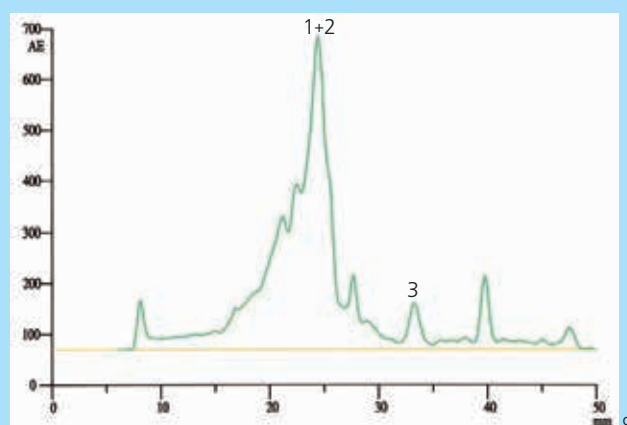
A 25-step gradient for a regular 200 µm HPTLC plate using 3 mm distance increments, takes about 4 hours, all intermediate drying and reconditioning steps included. Reducing the number of steps to 20 lowers the total time to about 2.5 hours. A reduction of AMD running times by up to 50% can be reached by using "extra thin" (100 µm) layers which require only 2 mm migration increments and shorter drying times.

A similar reduction in separation time is also possible with HPTLC LiChrospher®-Layers.

A feed supplement extract spiked with 50 ng/L ea. sulfamerazine (1), sulfadimidine (2) and dimetridazole (3) was used for chromatography. (1) and (2) differ only by one methyl group in the molecule.

Stage 1

Normal AMD universal gradient based on dichloromethane; preconditioning of the layer to slightly basic.



Result:

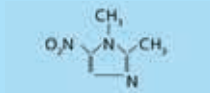
No separation of (1) from (2); (3) reasonably separated.

Varying the gas phase to acidic gave a better separation of (3), but no significant change regarding (1) and (2).

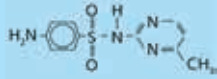
Conclusion:

Change of selectivity seems necessary, e.g. changing the base solvent.

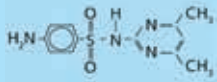
Sulfamerazine



Sulfadimidine



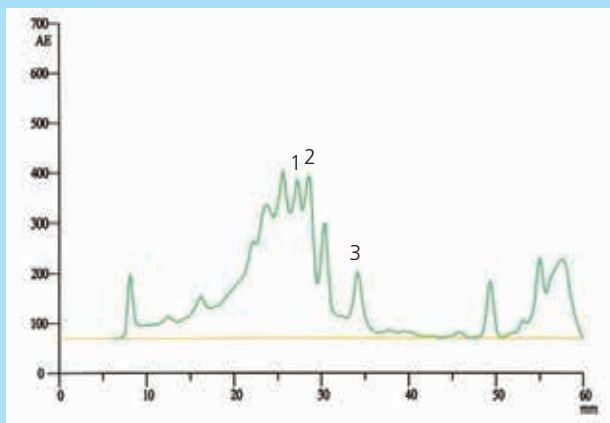
Dimetridazole



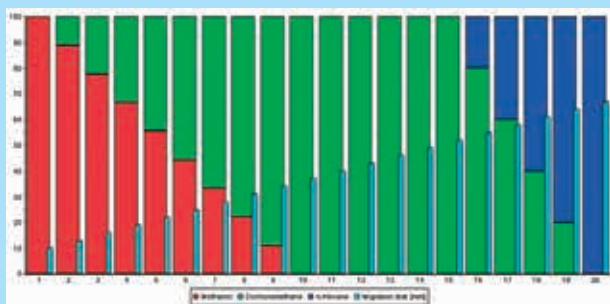
The complete method for determination of sulfonamides and tetracyclines in animal feed by AMD is described CAMAG Application note A-29.

Stage 2

Universal gradient based on diisopropyl ether



11



12

Result:

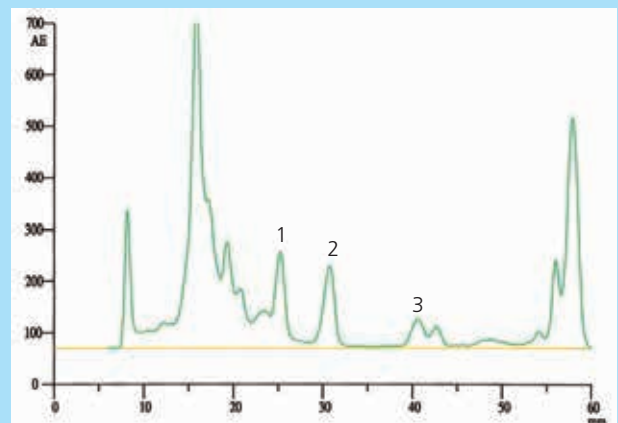
All three substances begin to separate, but resolution is insufficient.

Conclusion:

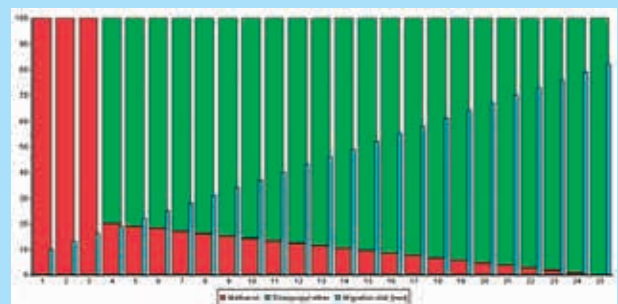
- Diisopropylether is a suitable base solvent for this task
- The gradient has to be much shallower
- The non-polar part, i.e. gradient from diisopropyl ether to hexane, is not needed.

Stage 3

From these observations the gradient depicted below was defined. Three isocratic development steps with methanol were found to be necessary to ensure that no substance remains stationary at the line of sample application (origin) where it could entrap analytes.



13



14

Result:

Good resolution of all three components.

Ordering information

022.8860 **CAMAG AMD2-System** comprising chromatogram developing module, AMD 2 EquiLink and accessories; operating voltage 115V nominal (90–130V) or 230V nominal (180–260V) internal jumper selectable, 50/60 Hz.

Accessories include all electrical and pneumatic connecting elements, supply of sealing materials, HPTLC plate positioning device, and small ancillaries

Dimensions: 430 × 500 × 550 mm (W × D × H)

Weight: 31 kg

027.6300 **winCATS License** including 1 year Internet Update Service

022.8880 **Oil-sealed rotary vane pump RV3** (Edwards)

operating voltage 100–120 V or 220–240 V selectable, 50/60 Hz

Specifications:

Average pumping speed PNEUROP 3.3 m³/h

Ultimate partial pressure with gas ballast 0.8 mbar

Ultimate partial pressure without gas ballast 0.02 mbar

Motor power 0.25 kW

Noise level 48 dB(A)

Dimensions: 170 × 500 × 450 mm (W × D × H)

Weight: 24 kg

Notes:

For operating the AMD system, an external supply of compressed nitrogen or air (4 bars) is required; consumption: about 10 L/h.

If the AMD system is to be operated with a different type vacuum pump, note that a minimum average pumping speed of 3 m³/h according to PNEUROP is required and an ultimate partial pressure of < 10 mbar is reached.

 **WORLD LEADER IN
PLANAR CHROMATOGRAPHY**

CAMAG (Switzerland) · Sonnenmattstrasse 11 · CH-4132 Muttenz 1
Phone +41 61 467 34 34 · Fax +41 61 461 07 02 · info@camag.com

CAMAG (Germany) · Bismarckstrasse 27–29 · D-12169 Berlin
Phone +49 30 516 55 50 · Fax +49 30 795 70 73 · infoberlin@camag.com

CAMAG Scientific Inc. (USA) · 515 Cornelius Harnett Drive · Wilmington, NC 28401
Phone 800 334 3909 · Fax 910 343 1834 · tlc@camag.com

www.camag.com